Disruption of IFT results in both exocrine and endocrine abnormalities in the pancreas of *Tg737^{orp}* mutant mice

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While relatively ignored for years as vestigial, cilia have recently become the focus of intense interest as organelles that result in severe pathologies when disrupted. Here, we further establish a connection between cilia dysfunction and disease by showing that loss of polaris (*Tg737*), an intraflagellar transport (IFT) protein required for ciliogenesis, causes abnormalities in the exocrine and endocrine pancreas of the *Tg737^{orp}* mouse. Pathology is evident late in gestation as dilatations of the pancreatic ducts that continue to expand postnatally. Shortly after birth, the acini become disorganized, undergo apoptosis, and are largely ablated in late stage pathology. In addition, serum amylase levels are elevated and carboxypeptidase is abnormally activated within the pancreas. Ultrastructural analysis reveals that the acini undergo extensive vacuolization and have numerous ‘halo-granules’ similar to that seen in induced models of pancreatitis resulting from duct obstruction. Intriguingly, although the acini are severely affected in *Tg737^{orp}* mutants, cilia and *Tg737* expression are restricted to the ducts and islets and are not detected on acinar cells. Analysis of the endocrine pancreas in *Tg737^{orp}* mutants revealed normal differentiation and distribution of cell types in the islets. However, after fasting, mutant blood glucose levels are significantly lower than controls and when challenged in glucose tolerance tests, *Tg737^{orp}* mutants exhibited defects in glucose uptake. These findings are interesting in light of the recently proposed role for polaris, the protein encoded by the *Tg737* gene, in the hedgehog pathway and hedgehog signaling in insulin production and glucose homeostasis.

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Cilia are found on most cells in the mammalian body, and can be motile or immotile (primary cilia).¹ They play important roles in fluid movement, cell migration, and sensory reception.^{2,3} Multiciliated cells lining the trachea have a crucial function in mucociliary clearance, while ependymal cells lining the ventricles in the brain are involved in the transport of cerebrospinal fluid.⁴ Although much less is understood with regards to primary cilia in mammals, they have been implicated as mechanosensors in the kidney that detect fluid flow through the lumen of the collecting ducts.⁵ Furthermore, modified primary cilia in the nasal passages and eye are important in olfactory function and light reception.^{6,7} It is thought that cilia may also function in

chemoreception where they evaluate the local environment. A sensory role for cilia in mammals is further supported by the localization of numerous olfactory receptors, as well as somatostatin-3 (SST3) and serotonin (5-HT₆) receptors, to this organelle.^{3,8}

The mechanism of cilia assembly is highly conserved across all ciliated eukaryotes analyzed to date. First identified in *Chlamydomonas*, intraflagellar transport (IFT) describes the assembly of a large protein complex (IFT particle) at the base of the cilia (basal body) and its movement along the cilia axoneme.^{9–11} Anterograde and retrograde movement of the IFT particle is mediated through the action of the kinesin II (Kif3A, Kif3B, and Kap) complex and a cytoplasmic dynein motor protein, respectively. IFT function and the flagella play important roles in intracellular signal transduction, including mating and gamete activation through a cAMP-mediated pathway in *Chlamydomonas*.^{12,13} Mutations in several IFT proteins have now been described in lower eukaryotes such as *Trypanosomes*, *C. elegans*, *Chlamydomonas*, and

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Drosophila,^{11,14–19} while mutations in polaris (IFT88), wimple (IFT172), and the kinesin II (Kif3A and Kif3B) complex have been described in mouse.^{20–22} In all organisms, loss of an IFT protein results in similar defects where cilia are absent or severely stunted.

A recent set of data has implicated proteins that localize to cilia or basal bodies in the etiology of severe pathologies. In humans, these include cystic kidney disease-associated proteins polycystin-1, polycystin-2, and fibrocystin; mutations of which cause dominant and recessive forms of polycystic kidney disease (ADPKD and ARPKD).^{23–25} The BBS family of proteins, involved in Bardet–Biedl syndrome (BBS),^{26–28} and nephrocystin-1, inversin, and nephroretinin, which cause the pathology in nephronophthisis patients, are associated with mutations in cilia- or basal body-localized proteins.^{29,30} Interestingly, the pathologies in BBS and nephronophthisis are not restricted to the kidney, but can include obesity, diabetes, retinitis pigmentosa, chronic sinusitis, learning disabilities, hypertension, and infertility, suggesting that alterations in cilia/IFT function may have important roles in these pathologies that have yet to be explored.

In this regard, multiple mouse models for cystic kidney disease that have cilia abnormalities also exhibit pathology in the pancreas.^{31–34} Furthermore, pancreatic lesions have been reported in 10% of human ADPKD patients,^{35–39} and pancreatic fibrosis has been described in some patients with ARPKD.⁴⁰ The connection between cilia defects and pancreatic pathology has not been extensively explored nor is there a known role for cilia in pancreatic function.

The pancreas is a complex organ with both exocrine and endocrine functions that is derived from a common precursor of the primitive foregut.⁴¹ The endocrine compartment (islets) hormonally regulate glucose homeostasis while the exocrine portion (acinar cells, centroacinar cells, and ducts) functions in the production and release of digestive enzymes into the duodenum.⁴² From the limited characterization thus far, it appears that the exocrine compartment is the most profoundly affected in the cystic kidney disease models.^{43,44}

In addition to an association with disease pathology, cilia dysfunction has also been attributed to numerous developmental abnormalities. Human patients with Kartagener syndrome, along with mice that have mutations in either cilia assembly genes or in the polycystin-2 gene, exhibit *situs inversus* due to cilia defects on a gastrulation stage organizing center called the embryonic node.^{20–22,45–48} Abnormalities in neural tube closure and patterning have also been attributed to cilia/IFT dysfunction in mice.^{20,49} In the case of the neural tube, the defects are associated with changes in hedgehog signaling activity.⁴⁹

Interestingly, hedgehog signaling also plays an important role in the formation of the pancreas.^{50–53} Ectopic expression of sonic hedgehog (Shh) inhibits pancreas formation while loss of indian hedgehog

(Ihh) results in increased pancreas size with abnormal branching of ducts.^{50–52} In addition to developmental roles, hedgehog signaling is required for normal pancreatic physiology. Ihh, patched, and smoothened are expressed in the islets of the adult pancreas, and recent publications suggest that β -cells are a target for hedgehog signaling.^{53–55} Cyclopamine, a hedgehog signaling inhibitor, decreases both insulin secretion as well as insulin content of β -cells in cell culture.^{55–57} Furthermore, male mice heterozygous for patched mutations have impaired glucose homeostasis.⁵¹ Together, these results suggest that defects in the hedgehog signaling pathway may be a contributing factor to glucose intolerance, raising the question of whether loss of cilia or IFT may have a role in this process.

One of the better characterized IFT mutations is the transgene insertion-induced hypomorphic allele in *Tg737^{orpk}* mice.³¹ *Tg737* encodes the polaris (IFT88) protein whose function is required for cilia formation.⁵⁸ *Tg737^{orpk}* mutants are severely growth retarded and normally die within 2 weeks of birth, with very few surviving to 21 days of age (average age of death on the FVB background is approximately 5 days old). The cause of the early lethality in the *Tg737^{orpk}* mutants remains elusive, but it is likely due to a combination of pathologies in multiple tissues that include cystic lesions in the kidney, hydrocephalus, and hepatic and pancreatic abnormalities. Both the kidney and brain pathologies have been associated with stunted or absent cilia.⁵⁸

Here we analyze the association between pancreatic pathology and cilia defects. Our findings with regards to the exocrine pancreatic defects are similar to that recently reported by Cano *et al.*⁵⁹ The pathology includes cystic structures in the ducts, severe acinar cell apoptosis, acinar cell vacuolization with increased amylase release, abnormal intrapancreatic activation of carboxypeptidase, and elevated proliferation of the ductal epithelium associated with increased β -catenin expression. The phenotype is associated with defects in cilia on cells of the ducts and islets; however, the acinar cells were found not to have a cilium in either wild-type or mutant samples. These findings raise the questions as to why loss of *Tg737* in the islets and ducts causes such a severe pathology in the acini, which do not even express detectable levels of *Tg737*, and whether there are signaling events occurring between the cells in the exocrine pancreas that are required for maintenance of the acini.

Even more intriguing is our novel finding that loss of IFT function also results in abnormalities in the endocrine pancreas.⁵⁹ While the differentiation and distribution of the cell types in the islets of *Tg737^{orpk}* mutants appear normal, the mutants have reduced blood glucose after short-term fasting and have abnormalities in glucose homeostasis after a subsequent glucose challenge relative to wild-type controls. Since recent data have implicated IFT in

the hedgehog signaling pathway and hedgehog activity is known to be important in the regulation of insulin production and secretion,^{55,57} the results suggest that IFT or cilia may play a role in sensing and/or regulating blood glucose levels in mice.

Materials and methods

Mice

Tg737^{orp} animals were generated by a transgene insertion mutagenesis and were described previously.³¹ *Tg737^{A2-3β-Gal}* animals were generated by targeted mutagenesis in embryonic stem cells, which resulted in the insertion of a β -galactosidase reporter gene into the *Tg737* locus such that the endogenous *Tg737* promoter regulates its expression.²¹ All lines were maintained as heterozygous crosses on an FVB background in accordance with the IACUC regulations at the University of Alabama at Birmingham. Genotyping of tail prep DNA was performed as described previously.⁵⁸

Antibodies

Mouse monoclonal antiacetylated α -tubulin,⁶⁰ anti-glucagon, rabbit polyclonal anti- β -catenin, anti- α -catenin, and monoclonal rat anti-uvomorulin (E-cadherin) were purchased from Sigma (St Louis, MI, USA). Rabbit anti-phospho-histone H3 was purchased from Upstate (Lake Placid, NY, USA). Rabbit antiglucagon was obtained from Novocastra (England). Polyclonal antiinsulin antibody was purchased from ImmunoStar, Inc. (Hudson, WI, USA). Goat anti-amylase was purchased from Santa Cruz (Santa Cruz, CA, USA). Rabbit antisomatostatin was purchased from DAKO (Carpinteria, CA, USA). Rabbit anticarboxypeptidase A was acquired from Biogenesis (Poole, England). Rabbit polyclonal anti-PKD1 and anti-PKD2 antibodies were used as described.^{61,62}

Histological Analysis

To characterize the pathology of the pancreas, *Tg737^{orp}* mice and wild-type age-matched littermates ranging from embryonic day 17.5 (E17.5) to 56 days old were killed, the pancreas was dissected, fixed in neutral buffered formalin, embedded in paraffin, and sections were prepared and subjected to trichrome or hematoxylin and eosin (H&E) staining. Trichrome staining was achieved by deparaffinizing the tissue, then placing the sections in Bouin's fluid at 56°C for 1 h. The tissue sections were then rinsed and placed in working Weigert's iron hematoxylin stain for 10 min. After additional rinsing, sections were subjected to Biebrich scarlet-acid fuchsin solution for 10 min, rinsed, and placed in phosphotungstic-phosphomolybdic acid solution for 5 min. Sections were stained in aniline blue stain

solution for 10 min, placed in 1% acetic acid for 1 min, rinsed, and dehydrated through a series of anhydrous alcohols before mounting. H&E staining was accomplished as described previously.⁵⁸ Sections were analyzed using light microscopy.

β -Galactosidase Assays

β -Galactosidase assays for *Tg737* expression were conducted on sections through the pancreas isolated from the *Tg737^{A2-3β-Gal}* mouse²¹ as described by Taulman *et al.*⁵⁸ Briefly, pancreata were isolated from animals and fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for 1 h at RT, washed and infiltrated with 30% sucrose in PBS for 24 h, and snap frozen in Tissue Tek O.C.T. freezing compound (Sakura Finetek USA, Inc., Torrance, CA, USA). Sections (5 μ m) were cut and postfixed in 0.2% paraformaldehyde in 0.1 M piperazine-*N,N'*-bis-2-ethanesulfonic acid (PIPES) pH 6.5 and permeabilization in 0.05% NP-40/PBS for 15 min. Slides were washed once in PBS containing 2 mM MgCl₂ and incubated in X-gal staining solution (2 mM MgCl₂, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 1 mg/ml X-gal, 1 $\times$ PBS) at 37°C for 2 to 24 h. Sections were counterstained with nuclear fast red as described by the manufacturer (Vector Laboratories, Burlingame, CA, USA) and coverslips were attached using Permount. The specificity of the *Tg737* promoter-driven β -galactosidase reporter gene assay was demonstrated by its correlation with immunolocalization data.⁵⁸ Images were captured using MetaMorph imaging software and a Roper Scientific CoolSnap HQ CCD camera attached to a Nikon TE200 inverted microscope or a Coolpix 950 camera attached to a Nikon SMZ800 stereomicroscope.

For whole-mount β -galactosidase assays, pancreata from *Tg737^{A2-3β-Gal}* heterozygous and wild-type mice were placed in fixative (4% PFA, 0.05% Tween-20, 0.1 M PIPES pH 6.8) for 1 h at RT, washed twice for 10 min in PBS containing 2 mM MgCl₂, and then incubated in X-gal staining solution at 37°C for 1 to 2 days.

Immunofluorescence Microscopy

Pancreatic tissue was isolated from both wild-type and *Tg737^{orp}* animals and snap frozen in Tissue Tek O.C.T. compound (Sakura Finetek USA, Inc., Torrance, CA, USA). Samples were cut into 5 μ m sections, fixed with 4% PFA, 0.2% Triton X-100 in PBS for 10 min, then blocked for 10 min in 1% bovine serum albumen in PBS, and incubated with primary antibody diluted in blocking buffer at room temperature for 1 h. The tissue sections were washed four times in PBS, incubated for 45 min with FITC- and rhodamine-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) in blocking buffer. Nuclei were stained

with Hoechst No. 33528 (Sigma, St Louis, MO, USA) diluted 1:1000 in PBS, washed 3 times in PBS, then mounted in antifade mounting media (90% glycerol in PBS, 1 mg/ml *p*-phenylene diamine) and coverslips attached.

Apoptosis and Proliferation

The level of apoptosis was determined using the transferase-mediated dUTP nick end-labeling (TUNEL, Roche Diagnostics Co.) assay according to the manufacturer's protocol. The apoptosis index was determined by counting the amount of TUNEL staining nuclei seen in 10 representative fields at $\times 400$ magnification. Proliferation in pancreatic tissue was determined by immunostaining with anti-phospho-histone H3 (Upstate, Lake Placid, NY, USA). The proliferation index was measured by averaging the amount of anti-phospho-histone H3 stained cells in 10 fields at $\times 200$ magnification.

Western Blot Analysis

For pancreatic protein extracts, the pancreas was isolated from wild-type or *Tg737^{orp^k}* mutant littermates and dounce homogenized in a 50 mM Tris (pH 7.2) lysate buffer as described previously.⁶³ To analyze circulating amylase levels, whole blood was isolated from wild-type and *Tg737^{orp^k}* mutant mice, allowing the blood to incubate at RT for 2 h, then centrifuging at 14 000 r.p.m. to obtain serum. The protein concentration of all samples was determined using the DC protein assay kit (Bio-Rad, Hercules, CA, USA) as described by the manufacturer. Equivalent amounts of protein lysate (5–40 μ g) were resolved by electrophoresis on SDS-PAGE gels and transferred to nitrocellulose (Nitrobind; Micron Separations, Westboro, MA, USA). Filters were blocked in a 5% dry milk/0.2% Tween 20/PBS and Western blot analysis was performed with either rabbit polyclonal antibody against carboxypeptidase A (diluted 1:10 000 in blocking buffer shown above) or goat polyclonal antibody against amylase (diluted 1:2500 in blocking buffer shown above). Horseradish peroxidase (HRP)-conjugated anti-rabbit (Bio-Rad) and anti-goat antibodies (Santa Cruz) were diluted 1:1500 and incubated with filters for 1 h in blocking buffer. Filters were washed four times in PBS containing 0.2% Tween-20 and the HRP signal was detected using a Super-signal West Dura Chemiluminescence Kit (Pierce; Rockford, IL, USA).

Transmission Electron Microscopy

For transmission electron microscopy analysis, wild-type and *Tg737^{orp^k}* mutant animals ranging from E17.5 to postnatal day 5 were anesthetized and *in vivo* perfused with PBS followed by 4% PFA in

PBS. The pancreata were then removed and placed in a fixative (2% PFA, 2.5% glutaraldehyde in PBS) overnight at 4°C. Tissue was then rinsed in PBS buffer (pH 7.4), and post fixed in 1% osmium, 0.125% KFeCN, 0.1 M PBS buffer for 1 h. Tissue was then dehydrated through a graded series of alcohols to propylene oxide, and embedded in Spurr resin (Electron Microscopy Sciences, Fort Washington, PA, USA) at 70°C. Thin sections of pancreatic tissue were post contrasted with 2% uranyl acetate and Reynolds lead citrate. Sections were then analyzed on a Hitachi H7000 transmission electron microscope and images were captured at 75 kV.

Glucose Tolerance Test

In all, 14-day-old wild-type and *Tg737^{orp^k}* mutant mice were fasted for 4 h prior to the initial measurement of serum glucose levels. Glucose levels were determined using a OneTouch Ultra Glucometer (Lifescan, Milpitas, CA, USA) and OneTouch Ultra blood glucose test strips according to the manufacturer's instructions using blood collected from the tail vein. After initial measurement of glucose levels, 1.5 g glucose/kg body weight was injected intraperitoneally. Glucose levels were subsequently measured at 20, 80, and 140 min after injection.⁶⁴

Results

Pathology in the *Tg737^{orp^k}* Pancreas

The *oak ridge polycystic kidney* (*Tg737^{orp^k}*) mouse arose through a random insertional mutation event that resulted in partial loss of function of the *Tg737* gene.³¹ The pathology associated with the hypomorphic *Tg737* mutation includes cystic kidneys, cholangitis with biliary and bile duct hyperplasia, hydrocephalus, polydactyly, and abnormalities in the pancreas. The combination of these pathologies leads to severe growth retardation and early postnatal lethality normally within the first 2 weeks of birth (average age of death is 5 days old on the FVB background); however, the cause of death in these animals is unknown.

To better understand the biological role of *Tg737* and the pathological changes resulting from the partial loss of function in this gene, we have characterized the pancreatic phenotype associated with the *Tg737^{orp^k}* mutants.⁵⁹ At postnatal day 1, the overall size of the pancreas from mutant and wild-type embryos appears overtly similar when compared to the size of the liver, spleen, and body weight (Figure 1a, outlined). In contrast, the size of the pancreas in postnatal day 14 mutants (Figure 1b, outlined) is dramatically reduced relative to age- and sex-matched controls. Trichrome staining of sections through the *Tg737^{orp^k}* pancreas revealed that the pathology is associated with significant

Figure 1 Anatomical and histological analysis of the pathology in the *Tg737^{orpk}* pancreas. (a) In postnatal day 1 *Tg737^{orpk}* mutants, the size and gross anatomical appearance of the pancreas (outlined) is similar to its wild-type counterpart. (b) At postnatal day 14, there is a marked reduction in size of the pancreas in *Tg737^{orpk}* mutant mice compared to wild-type controls. (c, d) Trichrome staining of section through a day 14 *Tg737^{orpk}* mutant pancreas showing increased fibrosis in the exocrine pancreas as compared to wild-type, but no observable fibrosis within the islets. Original magnification was $\times 400$ for the panels in c and $\times 1000$ for panels in d. (e) At E17.5, H&E staining of the *Tg737^{orpk}* pancreas shows dilatation of ducts. Original magnification was $\times 400$. (f) This dilatation continued to expand at postnatal day 4. (g and h) In the few mutants that survive past 14 days of age, there is severe pancreatic pathology. Original magnification was $\times 400$. At postnatal day 21 (g) and day 56 (h), most of the pancreatic exocrine cells were ablated in *Tg737^{orpk}* mutants, while the islets located among adipose tissue are similar in gross appearance to those in wild-type controls. Original magnification was $\times 200$ for panels in g and $\times 100$ for panels in h.

amounts of collagen deposition and fibrosis, which occurred at both early and late stages of disease progression (Figure 1c, d). The fibrosis was evident within the acini, but not in the islets. Unlike many models of pancreatic disease, there was no histological evidence of a significant inflammatory response associated with the pathology, indicating that the *Tg737^{orpk}* mouse is not consistent with being a form of acute or chronic pancreatitis, but rather a model of severe noninflammatory pancreatic injury.

Histological analysis was conducted by light microscopy on H&E-stained sections at several stages to evaluate the course of disease progression. As early as E17.5, the lumens of the intercalated and intralobular ducts showed dilatations, while the morphology of the acini appeared relatively unaffected (Figure 1e). Similar pathological findings in

the ducts were seen in postnatal day 2 mutants, with only mild abnormalities detected in the acini at this level of analysis (data not shown). At postnatal days 4, 7, and 14, the luminal area of the ducts continued to expand and the ductal epithelium appeared flattened (Figure 1f; and data not shown). In addition, the acinar morphology became disorganized. While most *Tg737^{orpk}* mutants die within the first 1 or 2 weeks of birth, an occasional mutant will survive past this age. In two mutants that survived to 21 and 56 days of age, the pancreatic pathology was found to be particularly severe. In both animals, the acini were completely absent and the ducts were severely disorganized and difficult to detect (Figure 1g, h).

In contrast to the acini of the older mutants, the islets that are contained within adipose tissue appeared qualitatively normal with regards to

number and size. To determine if there were defects associated with the cell types in the endocrine compartment, sections of mutant and wild-type pancreas were analyzed by immunofluorescence using antibodies against insulin (β -cells), glucagon

(α -cells), somatostatin (δ -cells), and pancreatic polypeptide (PP-cells) (Figure 2a–c). The results of these analyses showed no obvious defects in the differentiation or spatial distribution of cell types in the islets.

Figure 2 Characterization of adult pancreatic endocrine tissue showed no observable changes in distribution of (a) α -cells (glucagon; green) or β -cells (insulin; red), (b) δ -cells (somatostatin; red), or (c) PP-cells (pancreatic polypeptide; red) as determined by immunofluorescence analysis.

***Tg737* Expression in the Pancreas**

Previous Northern blot analysis has shown that *Tg737* is expressed in most adult tissues with its highest levels detected in the lung, brain, testis, and kidney. Lower levels of *Tg737* are present in the liver, pancreas, and spleen, which likely reflects the lower abundance of ciliated cells in these tissues. To analyze the spatial pattern of *Tg737* expression in the pancreas, we utilized the β -galactosidase reporter gene incorporated into the targeting construct used to generate the *Tg737^{A2-3 β -Gal}* mutant mouse line.^{21,58} In newborn mice, whole-mount staining revealed low levels of β -galactosidase expression present throughout the pancreas, with elevated levels detected in the ducts (Figure 3a, b). No β -galactosidase activity was seen in wild-type controls (Figure 3c). Sections through the *Tg737^{A2-3 β -Gal}* heterozygous pancreas from newborn mice confirmed that the prominent β -galactosidase activity was present in the ducts (Figure 3d, arrow) and forming islets (Figure 3d, arrowhead). In adult mice, β -galactosidase activity was localized to the ducts and islets and was not detected in the acinar cells (Figure 3e). The β -galactosidase staining in the pancreas is detected as small dots of one or two per cell. The identity of these domains is unknown

but is typical of the staining seen in cells exhibiting a primary cilium. This has been, as reported previously, for the *Tg737^{A2-3 β -Gal}* mouse in the kidney and in regions of the brain.⁵⁸

Apoptosis and Proliferation in the Pancreas of *Tg737^{orp}* Mutants

At birth, the pancreas in *Tg737^{orp}* mutants appeared relatively normal with the exception of the mild dilatation of the ducts. However, shortly thereafter there was a dramatic reduction in the acinar cell population, with most of the acini being eliminated by postnatal day 21 (see Figure 1). To determine if the loss of the acini was occurring through an apoptotic mechanism, we analyzed sections through the mutant and wild-type pancreas prior to the onset of the severe pathology at postnatal day 3 by TUNEL (Figure 4a, b) staining and expression of activated caspase 3 (data not shown). The data indicated that there is a 5–10-fold increase in the number of apoptotic cells in the *Tg737^{orp}* mutant pancreata compared to wild-type controls (Figure 4a–d). Co-staining for amylase expression showed that a majority of the apoptosis occurred in the acini (Figure 4a) and not in the ducts or islets. The

Figure 3 The spatial distribution of *Tg737* expression in the pancreas. *Tg737* expression was analyzed using the *Tg737^{A2-3 β -Gal}* targeted allele. (a and b) In whole-mount staining of *Tg737^{A2-3 β -Gal}* heterozygous pancreas, β -galactosidase activity was detected in the ducts and islets. The image in (b) is a higher magnification of a pancreatic lobule showing the staining in the ducts (arrow) and islets (arrowhead). (c) There was no β -galactosidase activity observed in the wild-type controls. (d) Analysis of sections through the pancreas of day 1 *Tg737^{A2-3 β -Gal}* heterozygotes. β -Galactosidase staining is seen in cells of the ducts (arrow) and islets (arrowhead) as small dots in the cytoplasm. Similar staining has been reported previously in monociliated cells of the kidney and brain.⁵⁸ (e) Similar to that seen in day 1 *Tg737^{A2-3 β -Gal}* heterozygotes, the adult pancreas shows prominent staining in most, if not all, cells in the (i) islets and in the (d) duct epithelium, while there was no obvious *Tg737* expression seen in the acinar (a) cells of adult mice.

Figure 4 Apoptosis and proliferation is increased in the *Tg737^{orpK}* mutant pancreas. (a) TUNEL analysis (green) in wild-type and *Tg737^{orpK}* mutants showed that apoptosis in day 3 mutant mice was increased predominantly in amylase-positive acinar cells (red). (b) Graphic depiction of the relative apoptotic index that was determined across 10 regions of mutant and wild-type pancreas. (c) Cell proliferation was increased by roughly five-fold in day 3 *Tg737^{orpK}* mutants compared to wild-type pancreas as determined by anti-phospho-histone H3 immunostaining. (d) Graphic depiction of proliferation index determined for 20 regions of the wild-type and mutant pancreas. The proliferative cells were largely restricted to the ductal epithelium (data not shown).

apoptotic mechanism was further supported by the coexpression of activated caspase 3 in the cells positive for TUNEL (data not shown).

Although acinar cells were undergoing significant apoptosis in *Tg737^{orpK}* mutants, we also detected a significant increase in proliferating cells in sections of postnatal day 3 mutant animals as compared to wild-type controls (Figure 4c,d). This was determined by immunofluorescence using an anti-phospho-histone H3 antibody. In contrast to what is seen with apoptosis, the proliferating cells were located predominantly in the ducts and not in the islets and acini (data not shown).

Defects in *Tg737^{orpK}* Pancreas Result in Hyperamylasemia and Intrapancreatic Activation of Carboxypeptidase

Defects in exocytic pathways in the acinar cells and abnormal intrapancreatic activation of digestive enzymes can lead to disorganization and degradation of the acini and ducts similar to that seen in *Tg737^{orpK}* mutants.^{65–69} To begin evaluating whether there were defects in exocytosis in *Tg737^{orpK}* mutant mice, we compared levels of pancreatic and circulating amylase and determined if carboxypeptidase (CPA), a digestive enzyme released from acinar cell zymogen granules, was activated within the pancreas of the *Tg737^{orpK}* mutant at different stages in disease progression.

Amylase levels were determined in serum and pancreas extracts by Western blot analysis (Figure 5a,b). In serum from postnatal day 1 mutants, there was a marked increase in the levels of circulating amylase (Figure 5a). This occurs in the absence of any defects in creatinine clearance, indicating that the elevated amylase is not caused by the renal defects in *Tg737^{orpK}* mutants (data not shown). At later stages, circulating amylase dropped in both sets of animals, and was only evident in the mutants after prolonged overexposure, where it was slightly elevated (data not shown). In contrast to the circulating amylase, there were no differences between the levels of expression of amylase within pancreas of postnatal day 1 mutant and wild-type mice (Figure 5b). In 7-day-old mutant pancreas, the pancreatic amylase levels were markedly reduced compared to littermate controls and by postnatal day 14, pancreatic amylase was barely detectable. These data are consistent with the progressive loss of acini that becomes evident shortly after 14 days of age (see Figure 1).

To determine if the digestive enzymes are abnormally activated within the pancreas, processing of CPA was compared in wild-type and mutant mice through disease progression by Western blot analysis. The inactive precursor form of CPA (proCPA, 47 kDa) is normally detected in the wild-type pancreas, while the activated form of CPA (33 kDa) is not present. At postnatal day one, similar levels of proCPA are encountered in both mutant and

Figure 5 The *Tg737^{orpk}* mutant mouse shows markers of pancreatic injury and abnormal activation of digestive enzymes. (a) Circulating amylase levels were markedly increased in 1-day-old *Tg737^{orpk}* mutant mice compared to wild-type animals as determined by Western blot analysis. (b) In contrast to the circulating amylase, pancreatic amylase levels were roughly equivalent in day 1 *Tg737^{orpk}* mutant and wild-type mice. By days 7 and 14, pancreatic amylase levels dropped significantly in the *Tg737^{orpk}* mutants compared to wild-type controls, likely due to previous release of zymogen granules and the apoptotic loss of acini in the mutants. (c) Intrapaneatic activation of carboxypeptidase A was evident in *Tg737^{orpk}* mutants by Western blot analysis. In day 1 mutants, proCPA (arrow) was expressed at roughly the same levels as in wild-type controls. However, intrapancreatic activation of CPA was evident in the mutants as indicated by the detection of the 33 kDa form of activated CPA (arrowhead). At day 7, the levels of proCPA in mutants dropped, however, the activated form of CPA was still present.

wild-type samples; however, unlike the wild-type controls, the activated form of CPA is also evident in *Tg737^{orpk}* mutants (Figure 5c). By day 7, when signs of acinar abnormalities are present, the overall levels of proCPA are reduced in the mutants relative to controls. Despite this reduction in proCPA, the

activated form of CPA is still detected in the pancreas of 7-day-old mutants. Together, these results suggest that intrapancreatic activation of CPA is occurring in the mutants and that it may contribute to the loss of the acini.

Ultrastructural Analysis of Acini and Islets in *Tg737^{orpk}* Mutants

To better understand the defects in the exocrine cells of *Tg737^{orpk}* mutants, ultrastructural changes in the acini were evaluated in thin sections by transmission electron microscopy at E17.5 and postnatal days 1 and 5. At E17.5, there were no morphological differences observed in the acini from mutant and wild-type embryos (Figure 6a). Abnormalities in the mutants became evident by postnatal day 1, where nearly all of the zymogen granules in the acini exhibited a 'halo' appearance. The formation of halo-granules has been reported in acini shortly after secretin hyperstimulation as well as in models of pancreatitis induced by duct ligation.^{70,71} These data suggest that the acini in the *Tg737^{orpk}* mutants are active in the release of granule contents and is in agreement with the high levels of amylase detected in the circulation at this time point. In addition, there were cytoplasmic vacuoles detected in the mutant acini that are not present in the controls (Figure 6b) suggestive of cell injury. In 5-day-old mutants, the acinar cells exhibited even more extensive vacuolization, reduction in the general size of the zymogen granules, and dilatation of the rough endoplasmic reticulum (Figure 6c,d).^{70–73} In contrast to the acini, ultrastructural analysis of α - and β -cells in the islets showed no obvious morphological differences between wild-type and mutant pancreata at days 1 and 5 (Figure 6e and data not shown).

Presence of Cilia in the Pancreas and Cilia Defects in *Tg737^{orpk}* Mutants

Tg737 encodes a protein called polaris that is highly conserved across most eukaryotic organisms.^{21,58} Analysis of polaris in the mouse as well as its homologs in *C. elegans* (OSM-5), *Drosophila* (nompB), and *Chlamydomonas* (IFT88) reveal that its function is required for ciliogenesis as a component of the IFT pathway (reviewed in Kierszenbaum⁷⁴ and Scholey⁷⁵). To determine where cilia are located in the pancreas, we conducted immunofluorescence analysis of cryostat sections using antibodies to E-cadherin, acetylated- α -tubulin, amylase, glucagon, and somatostatin. From this analysis, it is evident that cilia are present on most cells lining the ducts (Figure 7a, left panels), as well as insulin (β -cells, Figure 7b, left panels), glucagon (α -cells, Figure 7c, left panels), and somatostatin (δ -cells, Figure 7d, left panels) producing cells. In contrast, no cilia were detected on any amylase

Figure 6 Ultrastructural analysis of *Tg737^{orpk}* mutant pancreas showed no obvious changes in representative islet cells; however, distinct morphological changes were evident in the acini shortly after birth. (a) Analysis of the pancreas at E17.5 by transmission electron microscopy revealed no detectable differences in wild-type (left) and *Tg737^{orpk}* mutant (right) acinar cell morphology. (b) However, in the acini from day 1 *Tg737^{orpk}* mutants (right) the zymogen granules exhibited 'halos' (arrow), which are not routinely seen in wild-type littermates. By postnatal day 5, *Tg737^{orpk}* mutant acinar cells present characteristics associated with severe pancreatic injury, including (c) extensive vacuolization (arrowhead), reduced size of zymogen granules, and (d) dilated endoplasmic reticulum. (e) Analysis of the islets in day 5 alpha (α)- and beta (β)-cells revealed no significant differences in morphology between wild-type and mutant pancreata.

Figure 6 Continued.

expressing cells (data not shown). Overall, the distribution of cilia correlates nicely with the β -galactosidase staining described above (see Figure 3) and with published data.⁷⁶

Previously, it was demonstrated that cilia were severely stunted in the collecting duct epithelium of the kidney and the ependymal cells lining the ventricles in the brain of *Tg737^{orp}* mutant.^{58,77} To determine if similar events were occurring in the duct and islet cells in the pancreas, we analyzed sections of the mutant pancreas for defects in the cilia by immunofluorescence (Figure 7a–d, right panels). While the cilia in the pancreas exhibited a wider degree of variability than revealed from our previous analysis in the kidney, in general, they were reduced in length and frequency in the pancreas of *Tg737^{orp}* mice compared to wild-type age-matched controls. When detected, the cilia were normally less than 0.5 μ m in length; however, there were also several examples in the mutants where cilia length was greater than that seen in the wild-type controls (data not shown).

Several other mouse mutations have been characterized that cause combinations of renal, hepatic, and pancreatic pathology similar to what is seen in *Tg737^{orp}* mutants.^{43,44,78} In many cases (such as polycystin-1, polycystin-2, fibrocystin, inversin, and cystin), the affected genes encode proteins that localize to renal cilia, suggesting that cilia dysfunction is a key factor in the pancreatic pathologies of these mutant mice.^{24,34,77,79} In contrast to *Tg737*, the loss of these proteins does not affect cilia assembly but more likely alters some aspect of their signaling activity. In addition, data have suggested that polycystin-2 accumulates at higher levels in the stunted renal cilium of *Tg737^{orp}* mutants.⁷⁷ To determine if similar effects are seen in the pancreas, the localization and expression of polycystin-1 and polycystin-2 were compared in mutant and wild-type pancreas at postnatal days 1 and 3. In contrast to previously reported results in the kidney, polycystin-2 was not detected in cilia in the pancreas but rather in cytoplasmic domains (Figure 8a). Furthermore, we found that expression of polycystin-2 in the mutant

Figure 7 Detection of ciliated cells in wild-type and *Tg737^{orpK}* mutant pancreatic tissue. Immunofluorescence analysis of sections of day 14 wild-type (left panels) pancreas indicate that cilia (arrows, acetylated α -tubulin, green) are present on (a) cells lining the ducts (E-cadherin, red), (b) β -cells (insulin, red), (c) α -cells (glucagon, red), and (d) δ -cells (somatostatin, red). In *Tg737^{orpK}* mutants (right panels), cilia were still present on these cell types but were generally decreased in length compared to wild-type controls.

Figure 8 Analysis of the ADPKD related cilia proteins in the pancreas of day 3 *Tg737^{orpK}* mutants. (a) In contrast to its localization in the kidney, polycystin-2 (PKD-2, red) was not detected in cilia (anti-acetylated tubulin, green) in the pancreas but rather in cytoplasmic domains. In *Tg737^{orpK}* mutants, PKD-2 expression was decreased compared to wild-type controls as determined by both immunofluorescence (compare a left and right) and (b) Western blot analysis. (c) Polycystin-1 (PKD-1, red) was detected in the basal bodies of wild-type pancreas (left panel) but not prominently along the cilia axoneme similar to that reported in renal epithelium. In contrast, in *Tg737^{orpK}* mutants, polycystin-1 was detected both in the region of the basal body and in the cilia (right panel, inset). The asterisks (*) denote cells shown in the insets with the staining removed for anti-acetylated α -tubulin (green).

pancreas was reduced relative to wild-type controls by both immunofluorescence and Western blot analysis as compared to the level of β -tubulin (Figure 8a, b). On Western blots, polycystin-2 antibodies detect two prominent isoforms, both of which are markedly repressed, but not absent, in the mutant samples.⁵⁹

In contrast to polycystin-2, polycystin-1 in the wild-type pancreas was predominantly localized around the basal bodies of both the ducts and cells of the islets (Figure 8c, left panel), similar to that seen in the renal epithelium. In mutants, polycystin-1 was still detected at the basal body; however, it was also seen along the axoneme, which was not characteristic of wild-type controls (Figure 8c, right panel).

***Tg737^{orpK}* Pancreatic Phenotype is Associated with Alterations in β -catenin Expression**

Several recent studies on cilia dysfunction in cystic kidney disease have revealed that the renal pathology is associated with alterations in β -catenin expression and localization.^{80,81} In addition, the

exocrine pancreas disorganization and apoptosis observed in mice lacking *mist1*, a basic helix-loop-helix transcription factor expressed in the acinar cells, is associated with defects in the adherens junctions and loss of catenin proteins.⁸² To explore the possibility that the pathology in *Tg737^{orpK}* mutants might also affect the adherens junctions, we analyzed the expression and localization of β -catenin by immunofluorescence and by Western blot analysis.

Immunofluorescence analysis of *Tg737^{orpK}* at postnatal day 9 mutant pancreas revealed that β -catenin expression was elevated specifically in the cytosol of dilated duct epithelium.⁵⁹ However, β -catenin expression was found to be decreased in the acini (Figure 9a), similar to that seen with *mist1* mutants.⁸² The general reduction in β -catenin expression is supported by Western blot analysis, which showed that there was an overall decrease in total β -catenin levels in the pancreas (Figure 9b). This decrease in β -catenin was evident in both 3- and 7-day-old mutant samples and thus occurred prior to the onset of the severe acinar cell apoptosis.

Figure 9 β -Catenin expression and epithelial junctions in the pancreas of *Tg737^{orpK}* mutant mice. (a) Analysis of β -catenin showed increased expression in the dilated pancreatic ducts of *Tg737^{orpK}* mutants (arrow, right panel) at postnatal day 9 compared to wild-type (left panel) controls. In contrast, the remainder of the mutant pancreas, including the acini, expressed lower levels of β -catenin. Insets show higher magnification images of the area indicated by the arrows. (b) This is supported by Western blot analysis of β -catenin in total protein extracts isolated from mutant and wild-type pancreas at postnatal days 3 and 7. Analysis of E-cadherin (c) revealed no significant changes in localization or expression levels between the wild-type and *Tg737^{orpK}* mutant pancreata. In addition, there were no changes detected for α -catenin or ZO1 (data not shown).

To determine if this was due to a general defect in adherens junctions, we also examined expression of E-cadherin, α -catenin, and ZO1, which subsequently showed no obvious changes in expression or localization (Figure 9c and data not shown).

Endocrine Function in *Tg737^{orpK}* Mutants

The expression of *Tg737* in the islets and recent data suggesting a connection between cilia/basal body defects and obesity-related diabetes in patients with BBS prompted us to evaluate whether impaired IFT in the *Tg737^{orpK}* mutants results in abnormalities in endocrine function involving glucose homeostasis.^{26,27,83} Owing to the severe pathology in the *Tg737^{orpK}* mutants and their early lethality, it was difficult to obtain large numbers of mutant mice to conduct this analysis. Thus, the data reported here is based on three sets of age-matched mice from each genotype (Figure 10).

To initiate the study, we fasted 14-day-old mutants and littermate controls for 4 h and then evaluated circulating glucose levels. Intriguingly, all three mutants had significantly lower glucose levels than controls (Figure 10, $\text{Mut}_{0\text{min}}$ Ave. = 120.7 ± 49 STD vs $\text{WT}_{0\text{min}}$ Ave. = 229 ± 48 STD; $P < 0.05$). We then evaluated the ability of *Tg737^{orpK}* mutants to respond to intraperitoneal (i.p.) injection of glucose

after fasting and measured the change in circulating glucose levels at 20, 80, and 140 min to determine the rate of return toward baseline levels (Figure 10). At 20 min after injection, blood glucose levels in wild-type and mutant controls rapidly increased and were not significantly different between genotypic classes ($\text{WT}_{20\text{min}}$ Ave. = 485 vs $\text{Mut}_{20\text{min}}$ Ave. = 427). At 80 min post-injection, the blood glucose levels in wild-type mice had nearly returned to fasting levels and was well below fasting levels at 140 min ($\text{WT}_{0\text{min}}$ Ave. = 229 vs $\text{WT}_{140\text{min}}$ Ave. = 130). In contrast, blood glucose levels in the mutants remained significantly elevated at 140 min ($\text{Mut}_{0\text{min}}$ Ave. = 121 vs $\text{Mut}_{140\text{min}}$ Ave. = 391; $P < 0.05$). For the single set of mice analyzed after 200 min, the mutant still had not returned to near fasting levels ($\text{Mut}_{200\text{min}}$ = 271) and remained well above the wild-type control ($\text{WT}_{200\text{min}}$ = 116). Although these data are based on a relatively small cohort of samples, it demonstrates a potentially important role for cilia or IFT in sensing and/or regulating circulating glucose levels in mice.

Discussion

Cilia have recently become the focus of intense research interest due to their association with

Figure 10 Endocrine pancreas defects in *Tg737^{orp}* mutant mice. Day 14 mutant and wild-type mice were fasted for 4 h and serum glucose levels were analyzed at indicated times after i.p. injection of glucose ($n=3$ sets of mice). Prior to glucose challenge, the fasting levels of serum glucose were found to be significantly lower in mutants than in controls ($P<0.05$). In addition, mutants were unable to regulate effectively blood glucose levels after glucose challenge. Mutants maintained elevated levels of blood glucose at 140 and 200 min after initiation of the challenge by which time the wild-type mice had blood glucose levels below that at fasting (at 140 min, $P<0.05$).

disease and developmental abnormalities. Here we further demonstrate the connection between cilia dysfunction and disease pathogenesis in the *Tg737^{orp}* mutant mouse. The *Tg737^{orp}* mouse is one of only a few mutations in an IFT protein in mammals and is unique in that it is a hypomorphic mutation. This has allowed homozygous mutants to survive past the early embryonic lethality common to the other cilia mutants and has provided the opportunity to evaluate the importance of cilia in normal tissue physiology and disease.

Tg737^{orp} mutants develop a complex pathology with cystic lesions in the kidneys, cholangitis with biliary and bile duct hyperplasia, hydrocephalus, and skeletal patterning defects. In addition, the *Tg737^{orp}* mutants exhibit severe pathology in the exocrine pancreas. Furthermore, the data reported here also indicate that impaired IFT or cilia function results in physiological defects in the endocrine pancreas that are associated with the regulation of glucose homeostasis.

Pathological findings in the *Tg737^{orp}* mutants were evident in late gestation, when the lumens of the intercalated ducts become abnormally dilated. These ducts continue to enlarge and specifically express elevated levels of β -catenin. In addition, there is substantial interstitial fibrosis, and the acini become disorganized and undergo apoptosis, even-

tually being ablated at late stages of disease progression.⁵⁹ We further extended the analysis of the exocrine pancreas by analyzing the acini for possible ultrastructural defects at the level of transmission electron microscopy. This analysis revealed that the acini exhibited numerous 'halo-granules' and displayed extensive vacuolization. These ultrastructural defects are associated with elevated circulating amylase levels along with intrapancreatic activation of the digestive enzyme CPA. Halo-granules and elevated serum amylase have been reported previously in models of induced pancreatitis resulting from ductal obstruction or after hyperstimulation of the pancreas by secretin administration.^{70,71} The formation of these halo-granules has been attributed to fluid uptake by acinar cells and due to active granule secretion. Whether this pathology in the *Tg737^{orp}* mutants represents a defect in regulating zymogen granule release or is simply an indicator of severe acinar cell injury remains to be determined; however, unlike the models of pancreatitis, there is no sign of a significant inflammatory response in the *Tg737^{orp}* mutants. The reason for this lack of inflammatory cell infiltration is not known, but it has been demonstrated that apoptotic removal of acinar cells is able to attenuate the inflammatory response in several models of ductal obstruction.⁸⁴

Cilia were found on cells in the islets and ducts of the pancreas but were absent from the acini. This is in agreement with the spatial distribution of *Tg737* expression examined previously in Figure 3. Together, these data raise an intriguing question of how defects in cilia or loss of IFT in cells of the islets or ducts can have such a profound effect on the acini, which do not express *Tg737* nor do they exhibit a cilium. Several possible explanations could be envisioned. Defects in cilia function could have effects on ion transport in the ductal epithelium and reduce the rate of fluid secretion into the duct lumen, as proposed in the kidney. This would be similar to the pathology seen in the pancreas of CFTR mutant mice where defects in chloride transport in the duct epithelium results in lack of fluid secretion and premature activation of pancreatic digestive enzymes.^{85–87} Alternatively, there may be an unidentified signaling pathway between the ducts and acini required for acinar cell survival, maintenance, or regulation of zymogen granule release.

As seen in other tissues in the *Tg737^{orp}* mice, the cilia in the mutant pancreas were generally shortened compared to the wild types. This is similar to the data reported by Cano *et al*;⁵⁹ however, it should be noted that there were multiple examples in the mutant pancreas where cilia length was markedly increased. Cilia length is dynamic in the pancreas and appears to be related to duct size. Elongated cilia have been reported in association with rat models of pancreatitis induced by duct ligation and have been attributed to an adaptive response caused

by an increase in intra-ductal pressure.⁷² While our data suggest that the ducts are dilated and disorganized, we do not have evidence suggesting that the ducts are obstructed or that increased ductular pressure is present in the mutants. Thus, the pathology may not be caused by simply a reduction in cilia length, but rather impaired cilia function or size control, or alternatively, a possible intracellular role for IFT unrelated to cilia.

The *Tg737^{orp}* mutant is one of several mouse models to study the etiology of PKD (reviewed in Guay-Woodford⁸⁸). Interestingly, many of these PKD models exhibited significant extrarenal pathology involving the pancreas. Similarly, mice with mutations in the polycystin-1 and polycystin-2 genes are associated with pancreatic abnormalities, and some human PKD patients with similar mutations develop pancreatic symptoms.^{35–39} Thus, it is interesting to note with regards to our characterization of cilia and the *Tg737^{orp}* pancreas phenotype, that both polycystin-1 and polycystin-2 have been detected in cilium of epithelial cells in the nephron.^{24,77} Furthermore, in the kidney of *Tg737^{orp}* mutants, it has previously been shown that impaired *Tg737* function results in an increase of polycystin-2 within the stunted cilium.⁷⁷ However, in contrast to the findings in the kidney, we did not detect polycystin-2 in the cilia of the pancreas. Rather, polycystin-2 was predominantly localized to cytoplasmic domains of the duct epithelium. While Cano *et al*⁵⁹ indicated that polycystin-2 is elevated specifically in the ducts of the pancreas, our data at both the level of immunofluorescence and Western blot analysis showed that polycystin-2 expression was reduced in the mutant pancreas. The cause of the discrepancy is uncertain, but may reflect differences in the genetic background on which the analysis was conducted, since this is known to impact disease presentation. In contrast to what was found with polycystin-2, the analysis of polycystin-1 revealed that it is predominantly localized to the basal body region in islet and duct cells of the wild-type pancreas as well as in faint domains in the cytoplasm. These results are similar to that described previously in the kidney.²⁴ In the *Tg737^{orp}* mutants, polycystin-1 was also present at the basal body; however, there were concentrated amounts located along the axoneme that were not seen in wild-type controls. These results raise the possibility that defects in IFT leads to inefficient retrograde transport of polycystin-1, which under normal conditions would be present at very low levels in the axoneme. Similar results have also been obtained with polycystin-1 in other tissues of the *Tg737^{orp}* mutants (Yoder, unpublished). The significance of polycystin-1 in the cilia remains unknown, but in the kidney, the polycystins are involved in a calcium signaling pathway that is elicited by fluid flow mediated deflection of the cilium on the apical surface of the collecting duct.²³ Defects in the polycystin-mediated signaling path-

way are associated with altered fluid secretion and nephron obstruction in dominant forms of polycystic kidney disease and thus may provide insights into the role of cilia on pancreatic duct epithelium (reviewed in Wilson⁸⁹).

The analysis of several cystic kidney disease mouse models, including the *Kif3a* mutants that have impaired IFT, has indicated that the pathology is associated with altered expression or localization of β -catenin in the kidney.^{80,81} Similarly, our analysis of the pancreatic phenotype in *Tg737^{orp}* mutants revealed that β -catenin levels are increased specifically in the cytosol of dilated duct epithelium.⁵⁹ However, we find that overall levels of β -catenin are repressed throughout the rest of pancreas. This is not an effect on general epithelial junction formation as seen in the *mist-1* model of pancreatic injury as the levels of α -catenin, ZO-1, and E-cadherin were not affected.⁸² Thus, the data indicate that the pathologies in the pancreas and kidney of IFT mutants may be associated with altered Wnt signaling activity as recently proposed.^{59,81}

In addition to the duct epithelium, cilia were also present on all cells localized to the islets. While there were no obvious morphological defects in the islets with regard to differentiation or spatial distribution of cell types in the *Tg737^{orp}* mutant pancreas, there were abnormalities detected in endocrine function. This was evident after a 4 h fast, where the mutants had a significant reduction in blood glucose levels compared to the wild-type controls. This finding indicates that there is an aberration in the ability of *Tg737^{orp}* mutants to respond to a reduction in blood glucose levels. While the regulation of glucose levels is extraordinarily complex, the reduced blood glucose levels in fasted mutants suggest the possibility that cilia or IFT defects impair glucagon production, release, or responsiveness. Similar effects on blood glucose levels are seen after fasting of mutants lacking the glucagon receptor, as well as in mice with mutations in the pro-hormone convertase (SPC2) that catalyzes the conversion of proglucagon to glucagons.^{90,91} However, unlike these mutants, there was no obvious indication of α -cell hyperplasia in the *Tg737^{orp}* mice. In addition, there is a significant neuronal role in glucose homeostasis. Falling blood glucose levels are detected by glucose responsive neurons in the hypothalamus and, through autonomic inputs, cause reduced β -cell insulin secretion and increased α -cell glucagon release by sympathetic and parasympathetic nerves (reviewed in Cryer *et al*⁹²). Since neurons are known to exhibit cilia, it is feasible that the defect resides outside of the pancreas and are of neuronal origin. Finally, with regards to the abnormal fasting glucose levels, there are data suggesting that intra-islet signaling by insulin or somatostatin also is involved in regulating glucagon release.^{93,94} Thus, loss of β - or δ -cell function may also contribute to abnormalities in

the α -cells. Further analysis of the fasting response will need to be conducted to distinguish between the many possibilities.

In addition to abnormal glucose homeostasis in fasting conditions, our results indicate that IFT or cilia may also play an important role in the β -cell-mediated response to a glucose challenge. In contrast to the wild-type controls, which returned to near fasting levels within 80 min of the glucose injection, the mutants still had dramatically elevated circulating glucose levels at 140 and 200 min after injection. This further raised the possibility that mutant cells have impaired glucose homeostasis, both in response to increasing or decreasing blood glucose levels. Thus, it will be of interest to analyze possible effects of IFT mutations on the localization and function of glucose transporters. Both glucose transporter-1 (GLUT-1) and glucose transporter-2 (GLUT-2) have been detected on the cilia of ependymal cells in the brain where they are thought to function as part of a brain glucose sensor.⁹⁵

The finding that *Tg737^{orpK}* mutants have abnormalities in glucose homeostasis is intriguing for several additional reasons. First, defects in cilia and basal body-related proteins have recently been associated with BBS that develop obesity and diabetes as part of the pathology.^{26,27,83} Second, the loss of *Tg737* function disrupts the hedgehog signaling pathway during development, and hedgehog signaling is known to play an important role in the regulation of insulin production in β -cells.^{49,55} The hedgehog family of proteins signal through the transmembrane protein smoothened, which is repressed by the transmembrane protein patched in the absence of the hedgehog ligand. Hedgehog, smoothened, and patched are expressed in the islets and mutations in this pathway result in congenital pancreatic malformations and have been associated with pancreatitis.^{50,54} In addition, patched heterozygous male mice exhibit similar abnormalities in glucose homeostasis as we report here for *Tg737^{orpK}* mutants. This discovery has led to the speculation that defects in hedgehog signaling may be a contributing factor to type II diabetes mellitus.⁵¹ From genetic studies, it was demonstrated that polaris functions downstream of the patched protein in the responding cell.⁴⁹ Together, the data raise the possibility that loss of IFT in the β -cells renders them insensitive to hedgehog signals and thus impairs their ability to induce or regulate insulin levels in response to altered blood glucose levels.

While our results showing glucose homeostasis defects associated with cilia defects is an intriguing hypothesis, it must be interpreted with caution based on the limited number of *Tg737^{orpK}* mutants that we were able to obtain for this study. The analysis of glucose homeostasis in the *Tg737^{orpK}* mice is greatly hindered by their compromised survival and extreme growth retardation characteristic of this mutant. In addition, the *Tg737^{orpK}*

mutants have significant pathologies in other tissues that may have effects on their ability to regulate serum glucose levels independent of the cilia abnormalities. For example, one of the functions of glucagon is to stimulate hepatic glucose production and subsequent release. Thus, it is also possible that the abnormalities in glucose homeostasis reflects a defect in hepatocytes; however, hepatocytes do not express detectable levels of *Tg737* nor do they have a cilium, thus it is difficult to predict a possible mechanism (Yoder, unpublished). To overcome these limitations, we are further pursuing these initial findings using a conditional mutation approach where IFT is being disrupted specifically in the β -cells. This will allow the analysis of glucose homeostasis to be conducted in the absence of the severe exocrine pancreas pathologies as well as extra pancreatic defects that plague the *Tg737^{orpK}* mutants.

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