SHORT COMMUNICATION

Novel Transgenic Mouse Model of Polycystic Kidney Disease

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Transmembrane protein 207 (TMEM207) is characterized as an important molecule for invasiveness of gastric signet-ring cell carcinoma cells. To clarify the pathobiological effects of TMEM207, we generated 13 transgenic mouse strains, designated C57BL/6-transgenic (Tg) (ITF-TMEM207), where the mouse *Tmem207* is ectopically expressed under the proximal promoter of the murine intestinal trefoil factor gene. A C57BL/6-Tg (ITF-TMEM207) mouse strain unexpectedly exhibited a high incidence of spontaneous kidney cysts with histopathological features resembling human polycystic kidney disease, which were found in approximately all mice within 1 year. TMEM207 immunoreactivity was found in noncystic kidney tubules and in renal cysts of the transgenic mice. The ITF-TMEM207 construct was inserted into *Mitf* at chromosome 6. Cystic kidney was not observed in other C57BL/6-Tg (ITF-TMEM207) transgenic mouse strains. Although several genetically manipulated animal models exist, this mouse strain harboring a genetic mutation in *Mitf* and overexpression of *Tmem207* protein was not reported as a model of polycystic kidney disease until now. This study demonstrates that the C57BL/6-Tg (ITF-TMEM207) mouse may be a suitable model for understanding human polycystic kidney disease. (*Am J Pathol* 2017, 187: 1916–1922; <http://dx.doi.org/10.1016/j.ajpath.2017.05.002>)

Transmembrane protein 207 (TMEM207) was originally identified by a large-scale effort termed the Secreted Protein Discovery Initiative, which attempted to find novel secreted and transmembrane proteins.¹ Subsequently, human TMEM207 was found to be overexpressed in many aggressive gastric signet-ring cell carcinomas. An interesting finding was that TMEM207 promotes tumor invasion, potentially through binding to a WWOX tumor suppressor molecule and weakening the molecule's tumor suppressive activity.² Furthermore, a mucinous adenocarcinoma of the colon was seen to strongly express TMEM207. Physiologically, TMEM207 expression is relatively limited in sebaceous gland cells, hair follicle bulge cells, and renal tubules. We hypothesize that ectopically expressed TMEM207 binds to WWOX and is related to gastrointestinal carcinogenesis.

To investigate the biological functions of TMEM207 in gastrointestinal tumors, we originally generated several C57BL/6-transgenic (Tg) (ITF-TMEM207) mouse strains,³ in which murine *Tmem207* is ectopically expressed under the proximal promoter (truncated approximately 200 bp) of the murine intestinal trefoil factor (*ITF*) gene (alias *Tff3*).

Unexpectedly, a C57BL/6-Tg (ITF-TMEM207) mouse strain exhibited a high incidence of spontaneous cysts in the kidney with histopathological features resembling those of various human polycystic kidney diseases (PKDs). In addition, kidney cysts were not observed in other C57BL/6-Tg (ITF-TMEM207) strains, and the ITF-TMEM207 construct appeared to be inserted into the microphthalmia-associated transcription factor (*Mitf*) gene at chromosome 6. Therefore, the inserted gene (ITF-TMEM207) may disrupt the existing *Mitf* gene.

MITF works as a master regulator of melanocyte development and function by regulating some differentiation genes, such as those involved in cell-cycle control, survival, motility, and infiltration.⁴ It has been shown that MITF has an oncogenic role in human melanomas and clear cell sarcomas.⁵

PKD is a hereditary disorder that is either autosomal dominant or recessive.⁶ Kidney and liver cysts manifest in

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various organs, and the condition progresses slowly, eventually resulting in renal insufficiency. PKD is the hereditary kidney disease with the highest frequency in adults.⁶ Genes responsible for autosomal dominant PKD include *PKD1* and *PKD2*, whereas *PKHD1* is responsible for autosomal recessive PKD; several genetically manipulated animal models exist relating to these genes.⁶ However, a mouse model in which *Mitf* is disrupted and *Tmem207* is overexpressed does not yet exist as a model of PKD. Therefore, we report this murine model that may be useful for studying human PKD.

Materials and Methods

Generation of the C57BL/6-Tg (ITF-TMEM207) Mouse Strain

The detailed procedure for generating the transgenic mouse strain with a C57BL/6 background and cassette vector has been reported previously.⁷ Briefly, to generate the C57BL/6-Tg (ITF-TMEM207) mouse, a plasmid containing the full coding region of the murine *Tmem207* gene, following the proximal promoter of the murine ITF gene (official name *Tff3*) was prepared. Briefly, a truncated 219-bp promoter region of murine ITF was obtained by PCR from C57BL/6 mouse genomic DNA using the following primers: 5'-AGTCTGCTTCTAGACTAGGTGTACAC-3' (sense) and 5'-GCCCTTTTATAGCCATGTGTTTGCTGGG-3' (antisense). First, the truncated promoter region was subcloned into a previously described cassette vector⁷ harboring a poly(A) signal, which was verified by sequencing. Subsequently, the entire coding region of murine *Tmem207* cDNA was amplified by RT-PCR from C57BL/6 kidney tissue RNA and was inserted behind the ITF promoter. Synthesis of the first strand of cDNA was primed with random hexamers using an RNA Long and Accurate PCR kit (TaKaRa, Ohtsu, Japan). Primers used to obtain *Tmem207* cDNA were 5'-ATCTGGGTACATCTTTCTTTTATAG-3' (sense) and 5'-CTGCTGCATCTGGATAAAAT-3' (antisense). After verifying the entire ITF-TMEM207 gene sequence, the construct was cut out with *Bgl*III and *Sph*I restriction enzymes, and the construct fragment was microinjected into pronuclei of fertilized C57BL/6 oocytes using standard procedures. The resulting founder transgenic mouse was transferred to specific pathogen-free housing. Tail samples were digested with proteinase K (TaKaRa), and genotyping was performed by PCR to screen for transgenic founder animals as well as to perform routine genotyping. The PCR primers used for tail genotyping were as follows: 5'-AGTCTGCTTCTAGACTAGGTGTACAC-3' (sense) and 5'-ATACCAGCCATCAGGATATCGCTCGTC-3' (antisense).

The experimental protocol was approved by the Animal Care Committee of Kochi Medical School (Kochi, Japan) and Gifu University Graduate School of Medicine (Gifu, Japan).

Animals

To quantify cyst size, 35 C57BL/6-Tg (ITF-TMEM207) mice (17 males and 18 females, 22 homozygotes and 13 heterozygotes, from 1- to 2-year-old mice) were examined. Similarly, to analyze the kidney size and weight, C57BL/6-Tg (ITF-TMEM207) mice [from 8- to 16-week-old mice (five males and five females)], >1-year-old mice (five males and five females, homozygotes only), and 10 wild-type mice (10-week-old mice, five males and five females) were examined.

And then, for research on age of onset of cyst formation (time course study of evolution of the cysts in mice), C57BL/6-Tg (ITF-TMEM207) mouse (homozygote) was divided into three groups: approximately 1-month-old mice ($n = 2$; one male and one female), 2- to 3-month-old mice ($n = 2$; one male and one female), and 4- to 5-month-old mice ($n = 2$; one male and one female). For each group, renal tissue sections were observed in detail. Serum creatinine concentration of C57BL/6-Tg (ITF-TMEM207) mice [from 8- to 16-week-old mice (two males and two females)], >1-year-old mice (two males and two females, homozygotes only), and six wild-type mice (16-week-old mice, three males and three females) were examined to analyze renal function. Serum creatinine concentration was determined using the LabAssay Creatinine (Wako, Osaka, Japan). These mice survive for at least 12 months.

Immunohistochemical Staining

The detailed procedure for immunohistochemical staining, including the preparation⁸ and characterization of the rabbit antibody specific to TMEM207² that can detect both human and murine TMEM207 protein, has been described previously.² A conventional mouse monoclonal antibody to the synthetic peptide VNYNDQHPNGW (amino acid residues 40 to 50 of TMEM207) was generated in our laboratory.

Tissues were immunostained with antibodies using the ImmPRESS polymerized reporter enzyme staining system (Vector Laboratories, Burlingame, CA), as previously reported.⁹

Genome Walking

The Universal GenomeWalker Kit (TaKaRa) and the APAGene GOLD-RT Genome Walking Kit (Bio S&T, Montreal, QC, Canada) were used to identify the inserted gene region of the ITF-TMEM207 construct. The procedures were performed according to the manufacturer's protocol.

RT-PCR and Quantitative Real-Time RT-PCR

RT-PCR was performed as previously described.^{2,9} Total cellular RNA was prepared from cell lysates using RNeasy (Ambion, Austin, TX). cDNA synthesis from total RNA and the subsequent PCR was performed using the RNA Long and Accurate PCR kit (TaKaRa). The procedure was performed according to the manufacturer's protocol. The primer sets used

in this study were as follows: 5'-GCTAAAAGCCTGGTGT-GCTGGCCGTCAGTGAGGG-3' (sense) and 5'-CAGCTGGCTCTACCAGAGTGCATCTTCATC-3' (antisense) for polycystin-1, and 5'-ACCACAGTCCATGCCATCAC-3' (sense) and 5'-TCCACCACCCTGTTGCTGTA-3' (antisense) for glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). The PCR-amplified products were electrophoretically separated on a 1% agarose gel.

Real-time PCRs were performed using the SYBR Green reaction kit, according to the manufacturer's instructions (Roche Diagnostics, GmbH, Mannheim, Germany) in a Light-Cycler (Roche Diagnostics). cDNA (2 μ L each) was diluted to a volume of 20 μ L, with the PCR mix containing a final primer concentration of 0.2 pmol.

The following primers were used for real-time RT-PCR: real-time quantitative PCR primer for mouse *Pkd1*, 5'-GCTAAGCTACACTTCTCTTTATCCG-3' (forward) and 5'-ACTTCTTGGCATCTTTCATCCCAC-3' (reverse); and *Gapdh*, 5'-ACCACAGTCCATGCCATCAC-3' (forward) and 5'-TCCACCACCCTGTTGCTGTA-3' (reverse).

Analysis of SYBR Green dissociation curves after completion of 40 PCR cycles revealed the presence of single amplicons for each primer pair. Samples were amplified as follows: one cycle at 95°C for 10 minutes, 40 cycles at 95°C for 10 seconds, and 60°C for 30 seconds. The expression of each target gene was analyzed by the $2^{-\Delta\Delta CT}$ method of Livak and Schmittgen¹⁰ using the LightCycler system (Roche, Mannheim, Germany). The ΔC_T values were normalized to *Gapdh* for each triplicate set in both the wild-type (control) and C57BL/6-Tg (ITF-TMEM207) mouse group. The values for the C57BL/6-Tg (ITF-TMEM207) mouse group were then calculated for each target gene as the fold change relative to the mean values for the wild-type group (control; set to 1.0).

Western Blotting

Immunoblotting was performed essentially according to the modified method of Towbin et al,¹¹ as previously reported.² Briefly, mouse tissues were homogenized in a lysis buffer (CellLytic M cell lysis reagent; Sigma-Aldrich, St. Louis, MO), on ice for 30 minutes. Then, these total cell lysates were mixed 1:1 with 2 $\times$ SDS sample buffer [4% SDS, 20% glycerol, 0.12 mol/L Tris (pH 6.8), 0.2 mol/L dithiothreitol, and 0.02% bromophenol blue], incubated for 5 minutes at 95°C, and subjected to SDS-PAGE.

The separated proteins were transferred onto polyvinylidene difluoride membranes (Millipore Co, Bedford, MA). After blocking with bovine serum albumin, membranes were incubated with an anti-Mitf polyclonal antibody (Bioss Antibodies Inc., Woburn, MA) or anti-polycystin-1 antibody (Bioss Antibodies Inc.). Immunoreactivity was assessed using a Western blotting detection kit (Promega, Madison, WI). The immunoblot band was quantified by densitometry using LI-COR C-DiGit Blot Scanner imaging software version 3.1 (LI-Cor Biosciences, Lincoln, NE) and was normalized to the *Gapdh* band.

Results

Kidney Pathology in the C57BL/6-Tg (ITF-TMEM207) Mouse Strain

The typical histopathological findings of the renal cysts are shown in Figure 1A. Renal cyst morphology consisted of a somewhat large simple cyst, multiple small cysts, and dilated tubules (Figure 1B). However, the renal cysts exist in both the cortex and the medulla; these cysts predominantly exist in the corticomedullary junction. The cysts are lined by a single layer of cuboidal to columnar to flat epithelium (Figure 1C). The cysts appear empty or filled with pale acidophilic material or fresh blood. Occasionally, cystic dilation of the Bowman space around glomeruli (Figure 1D) and dilation of the loop of Henle (Figure 1E) are observed. In intervening kidney tissue, focal lymphocytic infiltrate (Figure 1F), mild interstitial fibrosis, and tubular atrophy (Figure 1G) are observed. But, calcified deposits, renal cell carcinoma, thickening and lamination of tubular basement membranes, and glomerular and vascular sclerosis are not observed.

The histopathological findings of multiple small liver cysts are shown in Figure 1K, pancreatic cysts are shown in Figure 1L, and multiple pancreatic small cysts are shown in Figure 1M.

High Renal Cyst Frequency in the C57BL/6-Tg (ITF-TMEM207) Mouse Strain

Renal cysts were found in 77.1% (27 of 35 mice) of approximately 12-month-old heterozygous and homozygous mice. Some mice with renal cysts in this study also possessed small liver small cysts (20 mice) and pancreatic cysts (seven mice).

Quantification of cyst size is shown in Figure 2A. The majority of cyst size is 2.0 to 0.5 mm. No significant differences in their sex and whether they were homozygotes or heterozygotes were found. The C57BL/6-Tg (ITF-TMEM207) mice survival is >2 years. Kidney size, kidney weight, and creatinine were comparable in wild-type and C57BL/6-Tg (ITF-TMEM207) mice (Table 1). In contrast, in the C57BL/6-Tg (ITF-TMEM207) mice, so far observed big cyst in the kidney was rare and macroscopically there was no significant change in the surface of the kidney. However, on the cut surface, macroscopically mild expansion of the renal pelvis was observed.

In wild-type mice (approximately 10 weeks old), kidney sizes were 1.07 ± 0.025 cm (male) and 0.79 ± 0.14 cm (female). Kidney weights were 194.5 ± 6.28 mg (male) and 151.7 ± 5.95 (female). In 8- to 16-week-old C57BL/6-Tg (ITF-TMEM207) mice, kidney size and kidney weight were slightly increased. Kidney sizes were 1.12 ± 0.0036 cm (male) and 1.30 ± 0.16 cm (female). Kidney weights were 208.9 ± 8.58 mg (male) and 163.9 ± 7.90 (female). And, in >1-year-old C57BL/6-Tg (ITF-TMEM207) mice, kidney size and kidney weight were slightly increased compared

Figure 1 Representative histopathological findings of the kidney, liver, and pancreas that appeared in the C57BL/6-Tg (ITF-TMEM207) mouse and immunohistochemical staining using an antibody specific to TMEM207. **A:** Kidney of C57BL/6-Tg (ITF-TMEM207) mouse is enlarged with a simple cyst. **B:** Small polycystic kidney cysts in the kidney cortex. **C:** Dilated renal tubules and the cysts are lined by a single layer of cuboidal to columnar to flat epithelium. **D:** Cystic dilation of the Bowman space around glomeruli. **E:** Small polycystic kidney in the medullary and dilation of the loop of Henle. **F:** Focal lymphocytic infiltrate and mild interstitial fibrosis. **G:** Mild interstitial fibrosis and tubular atrophy. **H:** No significant immunoreactivity is found in the renal tubules of a wild-type mouse. **I:** TMEM207 immunoreactivity observed in the renal tubules of the C57BL/6-Tg (ITF-TMEM207) mouse. **J:** Cells lining kidney cysts of the C57BL/6-Tg (ITF-TMEM207) mouse were also stained with an antibody specific to TMEM207. **K:** Multiple cysts in the liver of the C57BL/6-Tg (ITF-TMEM207) mouse. **L** and **M:** Large and small pancreas cysts, respectively. **N** and **O:** Both renal tubules and secretion of human polycystic kidney disease show immunoreactivity with a specific murine monoclonal antibody to TMEM207. Original magnification: $\times 50$ (**A** and **L**); $\times 100$ (**B** and **K**); $\times 200$ (**C–J** and **M–O**).

with 8- to 16-week-old C57BL/6-Tg (ITF-TMEM207) mice. It is likely that kidney size and weight of the C57BL/6-Tg (ITF-TMEM207) mice slowly grew big and the color gradually grew more whitish than wild-type mice. And a few differences in kidney weight and size between male and female C57BL/6-Tg (ITF-TMEM207) mice were found.

Age of onset of cyst formation [time-course study of evolution of the cysts in homozygote C57BL/6-Tg (ITF-TMEM207) mouse] is shown in Figure 2B. In 1-month-old C57BL/6-Tg (ITF-TMEM207) mice ($n = 2$; one male and one female), there was no significant change. But, in 2- to 3-month-old C57BL/6-Tg (ITF-TMEM207) mice ($n = 2$; one male and one female), small kidney cysts begin to be observed. Most cysts were approximately 0.5 mm and composed of dilated tubules. And then, in 5- to 6-month-old C57BL/6-Tg (ITF-TMEM207) mice ($n = 2$; one male and one female), cyst size was larger and cyst number was

higher. When it comes to this time, cystic dilation of the Bowman space around glomeruli and dilation of the loop of Henle also begin to be observed.

To analyze renal function, serum creatinine concentration of the C57BL/6-Tg (ITF-TMEM207) mice (homozygotes only) and wild-type mice were examined. Serum creatinine concentration was determined using the LabAssay Creatinine (Wako, Osaka, Japan). In wild-type mice (approximately 16 weeks old), serum creatinine concentrations were 0.28 ± 0.027 mg/dL (male) and 0.10 ± 0.012 mg/dL (female). In 8- to 16-week-old C57BL/6-Tg (ITF-TMEM207) mice, serum creatinine concentrations were 2.31 ± 0.12 mg/dL (male) and 2.64 ± 0.50 mg/dL (female). In >1-year-old C57BL/6-Tg (ITF-TMEM207) mice, serum creatinine concentrations were 9.15 ± 0.61 mg/dL (male) and 1.90 ± 0.01 mg/dL (female). In only one transgenic mouse, significant aggravation of the renal function was

Figure 2 RT-PCR and Western blotting of several organs in the C57BL/6-Tg (ITF-TMEM207) mouse. **A:** Quantification of cyst size in the C57BL/6-Tg (ITF-TMEM207) mice by sex and homozygotes or heterozygotes. **B:** Age of onset of cyst formation in the C57BL/6-Tg (ITF-TMEM207) mice. One-month-old ($n = 2$; one male and one female), 2- to 3-month-old ($n = 2$; one male and one female), and 4- to 5-month-old ($n = 2$; one male and one female) mice. All are homozygotes. **C and D:** Decreased polycystin-1 expression in kidney and liver of the C57BL/6-Tg (ITF-TMEM207) mice. Lane 1, kidney; lane 2, spleen; lane 3, lung; lane 4, liver; and lane 5, brain. **E:** Quantification of *Pkd1* mRNA in wild-type mice and C57BL/6-Tg (ITF-TMEM207) mice. The ΔC_T values were normalized to *Gapdh* for each triplicate set in both the wild-type (control) and C57BL/6-Tg (ITF-TMEM207) mice. The values for C57BL/6-Tg (ITF-TMEM207) mice were then calculated for each target gene as the fold change relative to the mean values for the wild-type group (control; set to 1.0). **F:** Polycystin-1 protein in wild-type mice and C57BL/6-Tg (ITF-TMEM207) mice. The polycystin-1 protein band is detected in the kidney of wild-type mice. But, in the kidney of C57BL/6-Tg (ITF-TMEM207) mice, the expression of polycystin-1 was low. The immunoblot band was quantified by densitometry using LI-COR C-DiGit Blot Scanner imaging software (LI-Cor Biosciences, Lincoln, NE) and was normalized to the *Gapdh* band. This experiment was performed twice. **G:** Western blot using a rabbit polyclonal antibody against microphthalmia-associated transcription factor (*Mitf*). The *Mitf* protein band was detected in brains of wild-type mice. The observed band size in wild-type mice was 58 kDa (red arrow) but was absent in C57BL/6-Tg (ITF-TMEM207) mice. **H:** Transgene (ITF-TMEM207) was inserted into a basic helix-loop-helix leucine zipper lesion of the *Mitf* gene on chromosome 6. Original magnification, $\times 100$ (**B**).

observed, whereas aggravation of the renal function also was observed with other transgenic mice.

Exogenous *Tmem207* Expression in Renal Cysts of the C57BL/6-Tg (ITF-TMEM207) Mouse Strain

Although *ITF* is specifically expressed in intestinal goblet cells, the proximal promoter of *ITF* used in this study¹² is not sufficient to recapitulate the exquisite tissue- and cell-specific expression of the native *ITF*, but rather allows gene expression in various tissues, including the gastrointestinal tract, as it lacks a goblet cell silencer inhibitor element.¹³ Therefore, we further examined whether *Tmem207* was expressed in kidneys of C57BL/6-Tg (ITF-TMEM207) mice and their wild-type littermates. Immunohistochemical staining using a rabbit polyclonal antibody specific to *TMEM207* demonstrated the expression of *Tmem207* in kidneys of the C57BL/6-Tg (ITF-TMEM207) mice.

TMEM207 (rabbit polyclonal antibody) immunoreactivity was observed in proximal convoluted tubules, but not in the glomerulus (Figure 1, H–J). No significant *Tmem207* immunoreactivity in wild-type mouse kidney tissues was found. *Tmem207* immunoreactivity was also found in the epithelium lining kidney cysts.

Lack of Polycystin-1 in the Kidney and Liver of the C57BL/6-Tg (ITF-TMEM207) Mouse Strain

All polycystic kidney disease cases contain a mutation in either *PKD1* or *PKD2*. In this C57BL/6-Tg (ITF-TMEM) mouse strain, expression of polycystin-1 in the kidney and liver was low or decreased, as demonstrated by RT-PCR and Western blotting (Figure 2, C and D). Quantitative RT-PCR also showed that the *PKD1* mRNA expression level is lower in the C57BL/6-Tg (ITF-TMEM) mouse than in wild-type mouse (Figure 2E). Immunoblotting was performed to

Table 1 Average Value of Kidney Size, Kidney Weight, and Renal Function (Creatinine) in Wild-Type and C57BL/6-Tg (ITF-TMEM207) Mice

		Transgenic mice	
Parameters	Wild-type mice	8–16 weeks	>1 year
Kidney size, cm			
Male	1.07 ± 0.025	1.12 ± 0.0036	1.32 ± 0.0036
Female	0.79 ± 0.14	1.30 ± 0.16	1.20 ± 0.00090
Kidney weight, mg			
Male	194.5 ± 6.28	208.9 ± 8.58	310.8 ± 23.68
Female	151.7 ± 5.95	163.9 ± 7.90	230.3 ± 13.74
Creatinine, mg/dL			
Male	0.29 ± 0.027	2.19 ± 0.12	9.15 ± 0.61
Female	0.10 ± 0.012	2.63 ± 0.50	1.89 ± 0.01

Data are expressed as means ± SD.

determine whether the C56BL/6-Tg (ITF-TMEM) mouse kidney expressed polycystin-1 again. The polycystin-1 protein band was detected in kidney of wild-type mice. But polycystin-1 protein was lower expressed in the C56BL/6-Tg (ITF-TMEM) mouse kidney rather than in the wild-type mouse (Figure 2F). The immunoblot band was quantified by densitometry using LI-COR C-DiGit Blot Scanner imaging software and was normalized to the Gapdh band. This experiment was performed two times. Quantitative band data (polycystin-1/Gapdh) were 0.032 (wild type) and 0.000707 [C57BL/6-Tg (ITF-TMEM207) mice]. Second quantitative data were 0.072 (wild type) and 0.00108 [C57BL/6-Tg (ITF-TMEM207) mice]. We hypothesized that low polycystin-1 expression was likely influenced by the ectopic *Tmem207* expression in the current transgenic mice.

Chromosome Location of the ITF-TMEM207 Construct

A transgene often involves gain of function, but loss of function may occur if the transgene inhibits another gene. GeneWalking was subsequently performed to identify the gene insertion location of the ITF-TMEM207 construct, using the Universal GenomeWalker Kit (TaKaRa) and APAGene GOLD-RT Genome Walking Kits. The ITF-TMEM207 construct appeared to be inserted into *Mitf* at chromosome 6. Specifically, this transgene is inserted into a basic helix-loop-helix leucine zipper region of *Mitf* (Figure 2H).

Immunoblotting was performed to determine whether the C56BL/6-Tg (ITF-TMEM) mouse tissue expressed *Mitf* protein. The *Mitf* protein band was detected in the brain of wild-type mice. *Mitf* protein was expressed at a lower level in the C56BL/6-Tg (ITF-TMEM) mouse brain than in the wild-type mouse (Figure 2G).

Considering that renal cysts and liver cysts were not found in other C57BL/6-Tg (ITF-TMEM207) mouse strains, ectopic *Tmem207* expression and *Mitf* disruption were associated with the occurrence of renal cysts in the present transgenic mice.

Expression of TMEM207 in Human Polycystic Kidney Disease

Subsequently, immunohistochemical staining was performed to examine TMEM207 expression in kidney specimens of human polycystic kidney disease. We used a specific murine monoclonal antibody to TMEM207 generated in our laboratory. Notably, the result of immunohistochemical staining using murine monoclonal antibody to TMEM207 was similar to that using the previously used rabbit polyclonal antibody to TMEM207.² A representative staining is shown in Figure 1, N and O. Immunoreactivity with the anti-TMEM207 antibody was found in three of the five kidney specimens of human polycystic kidney disease. Immunoreactivity was found in renal tubules and secretion in specially dilated renal tubules and flat epithelium. In contrast, there was no significant staining in the interstitial tissue and glomerular tissue of kidney specimens of human polycystic kidney disease. We concluded that TMEM207 was expressed in dilated renal tubules of human polycystic kidney disease.

According to a previous study,¹⁴ TMEM207 expression is related to the mucinous phenotype of colorectal cancer and signet-ring cell carcinoma of gastric cancer. In other words, it is supposed that TMEM207 is related to enhancement of the secretion ability. Similarly, in the kidney of human polycystic kidney disease, it is guessed that the expression of TMEM207 reflects the enhancement of the secretion ability.

Discussion

In this study, we described a new transgenic mouse strain, C57BL/6-Tg (ITF-TMEM207), in which spontaneous renal cysts formed with a high incidence. Morphopathological features of these renal cysts are similar to features of human renal cysts that occur in polycystic kidney disease, which were histopathologically characterized as dilated tubules. Several of the mice also exhibited liver cysts and pancreatic cysts. Therefore, we speculate that the present mouse model will be useful for analyzing the pathological characteristics of human polycystic kidney disease.

A genome walking assay showed that the transgenic plasmid construct was inserted into *Mitf* at chromosome 6 in the C57BL/6-Tg (ITF-TMEM207) strain. We could not observe renal cysts in the other 13 transgenic mouse strains. Furthermore, the expression of polycystin-1 in the kidney and liver was low or decreased in this mouse strain.

Taken together, the exogenous expression of *Tmem207* in renal tubules and renal cyst wall cells, along with a disrupted *Mitf* gene, may be responsible for the formation of the renal cysts observed in this study. *Tmem207* expression in renal tubules is likely to be important for renal cyst development. Because the present study also revealed novel promoter activity of the truncated *ITF* sequence, we initially expected that the ubiquitous enforced expression of

Tmem207 would be observed in the gastrointestinal tract because this truncated promoter lacked a goblet cell silencer inhibitor element. This truncated *ITF* sequence induced exogenous Tmem207 expression in renal tubules (Figure 1, H and I). We observed exogenous Tmem207 expression in renal tubules of other transgenic mouse strains. Therefore, the truncated *ITF* promoter used in this study may be useful for expressing exogenous proteins in the renal tubules and studying their biological role. Moreover, it is likely that disrupted *Mitf* expression in renal tubules may be the crucial factor for renal cyst development because the c-Met receptor tyrosine kinase is a multifaceted regulator of growth, motility, and invasion in a number of lineages *in vivo*, and c-Met is a direct transcriptional target of *Mitf*.¹⁵

In addition, it is possible that as the motion and function of renal tubule cilia-sensed urine flow decline, cilia dysfunction is connected to renal tubule dilation and renal cysts. Another possibility is that the affected gene in Waardenburg syndrome type 2 is responsible for establishing the collagen network that forms the basement membranes that support cells in the cochlea, retina, and kidney. On disruption of this gene, it is thought that the kidneys may become cystic. Approximately 85% of autosomal dominant PKD cases possess a mutation in *PKD1*, with the remaining 15% of cases having a mutation in *PKD2*.⁶ Because the C57BL/6-Tg (ITF-TMEM207) mice exhibit a phenotype that is similar to the phenotype of human PKD, it would be interesting to examine the relationship between TMEM207, PKD1, and MITF proteins.

Although this is an interesting possibility, there remain unanswered questions concerning the role of WWOX and *Mitf* in the pathogenesis of cyst formation.

Going forward, further studies should be required to elucidate the mechanism of functional link of TMEM207 with polycystin-1.

In contrast, more important, TMEM207 is expressed in dilated renal tubules of human polycystic kidney disease. Based on the analysis of previous studies,¹⁴ TMEM207 expression is related to enhancement of the secretion ability. In this regard, TMEM207 expression of human polycystic kidney disease may also help to explain, reflecting the enhancement of the secretion ability in the renal tubules.

In conclusion, the present study provides four important findings. First, the C57BL/6-Tg (ITF-TMEM207) mouse strain may be a novel model for understanding human PKD. Genes responsible for autosomal dominant polycystic kidney disease include *PKD1* and *PKD2*; *PKHD1* is responsible for autosomal recessive polycystic kidney disease. Other polycystic kidney models exist in rats and mice with mutated genes, such as *Nphp3*, *Tmem67* (alias *Mks3*), and *Invs*. However, a mouse strain harboring a genetic mutation in *Mitf* and overexpression of Tmem207 protein does not yet exist as a model of polycystic kidney disease.

Second, *Mitf*-mutated mice did not exhibit polycystic kidneys, but the overexpression of Tmem207, along with a disrupted *Mitf*, resulted in the down-regulation of polycystin-1

and exhibition of polycystic kidney disease characteristics. Third, the truncated *ITF* promoter may be useful for expressing exogenous genes in renal tubules. In addition, although needing further investigation, our results suggest a possible relationship between TMEM207, MITF, and PKD. Fourth, TMEM207 is expressed in dilated renal tubules of human polycystic kidney disease. TMEM207 may be molecularly targeted for the development of a new therapeutic approach for patients with polycystic kidney disease.

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