Transcription Factor 21 Is Required for Branching Morphogenesis and Regulates the Gdnf-Axis in Kidney Development

Shintaro Ide,1 Gal Finer,2,3 Yoshiro Maezawa,1 Tuncer Onay,3,4 Tomokazu Souma,3,4 Rizaldy Scott,3,4 Kana Ide,1 Yoshihiro Akimoto,5 Chengjin Li,6 Minghao Ye,3,4 Xiangmin Zhao,2,3 Yusuke Baba,1 Takuya Minamizuka,1 Jing Jin,3,4 Minoru Takemoto,1,7 Koutaro Yokote,1 and Susan E. Quaggin3,4

Due to the number of contributing authors, the affiliations are listed at the end of this article.

ABSTRACT

Background The mammalian kidney develops through reciprocal inductive signals between the metanephric mesenchyme and ureteric bud. Transcription factor 21 (Tcf21) is highly expressed in the metanephric mesenchyme, including Six2-expressing cap mesenchyme and Foxd1-expressing stromal mesenchyme. Tcf21 knockout mice die in the perinatal period from severe renal hypodysplasia. In humans, Tcf21 mRNA levels are reduced in renal tissue from human fetuses with renal dysplasia. The molecular mechanisms underlying these renal defects are not yet known.

Methods Using a variety of techniques to assess kidney development and gene expression, we compared the phenotypes of wild-type mice, mice with germline deletion of the Tcf21 gene, mice with stromal mesenchyme–specific Tcf21 deletion, and mice with cap mesenchyme–specific Tcf21 deletion.

Results Germline deletion of Tcf21 leads to impaired ureteric bud branching and is accompanied by downregulated expression of Gdnf-Ret-Wnt11, a key pathway required for branching morphogenesis. Selective removal of Tcf21 from the renal stroma is also associated with attenuation of the Gdnf signaling axis and leads to a defect in ureteric bud branching, a paucity of collecting ducts, and a defect in urine concentration capacity. In contrast, deletion of Tcf21 from the cap mesenchyme leads to abnormal glomerulogenesis and massive proteinuria, but no downregulation of Gdnf-Ret-Wnt11 or obvious defect in branching.

Conclusions Our findings indicate that Tcf21 has distinct roles in the cap mesenchyme and stromal mesenchyme compartments during kidney development and suggest that Tcf21 regulates key molecular pathways required for branching morphogenesis.
and subsequently into the Bowman’s capsule of the glomerulus, podocytes, and the nephron down to the distal tubule. The Six2-positive CM is surrounded by Foxd1-expressing stromal cells that are also derived from the MM and give rise to interstitial cells, mesangial cells, and pericytes. Disruption of any of these events results in congenital anomalies of the kidney and the lower urinary tract (CAKUT); these represent 20%–30% of all anomalies identified in the prenatal period in humans.

Cell type-specific basic helix-loop-helix transcription factors are key regulators of organ morphogenesis. Transcription factor 21 (Tcf21) (Pod1/Capsulin/Epicardin) is a class 2 basic helix-loop-helix transcription factor that shows robust expression in mesenchymal cells of the urogenital, respiratory, digestive, and cardiovascular systems throughout embryogenesis and is involved in epithelial–mesenchymal interactions in the kidney, lung, and other organs. In disease, Tcf21 is reported to function as a tumor suppressor and loss of Tcf21 expression and/or methylation of its promoter have been identified in a variety of lung and urogenital tumors. In other studies, Tcf21 has been linked to coronary artery disease, podocyte differentiation, diabetic kidney disease, and changes in adipose phenotype.

In the kidney, previous work has shown that Tcf21 null mutant mice are born with severely hypoplastic kidneys, and that although Tcf21 is exclusively expressed in the mesenchyme, in its absence, major defects in UB epithelial differentiation and branching are observed. Currently, it remains unclear what signaling pathways are controlled by Tcf21. In this study, we report that Tcf21 regulates branching morphogenesis by modifying the Gdnf-Ret-Wnt11 axis and provides data to support its pleiotropic functional roles that affect UB-MM-stromal crosstalk. Using the Cre-LoxP system we show that Tcf21 has distinct roles in Foxd1-positive stromal cells and Six2-positive CM cells; where selective deletion of Tcf21 from the stromal cells results in branching defects, a paucity of collecting ducts, and urine concentrating defects. On the other hand, Tcf21 deletion in the CM leads to defects in glomerulogenesis and massive proteinuria.

METHODS

Ethics Statement/Study Approval
All mouse experiments were performed in accordance with institutional guidelines for animal studies. All animal experiments were approved by the Animal Care Committee at the Center for Comparative Medicine of Northwestern University (Evanston, IL) or by the Chiba University Ethics Committee (Chiba, Japan).

Mice and Genotyping
Tcf21 lox/lox and Tcf21-LacZ mice were created as previously described. The Six2-eGFPCre and FoxD1-eGFPCre mice were kind gifts from Dr. Andy McMahon (University of Southern California), and are described elsewhere.

Real-Time PCR
Whole kidneys were dissected and preserved at −80°C after freezing in liquid nitrogen. They were treated by the homogenizer and QIA shredder (79656; Qiagen) and total RNA was extracted by using Pure link RNA minikit (12183025; Life Technologies). Reverse transcription was performed using Super Script III Reverse transcription (18080044; Invitrogen) according to the manuals. Quantitative real-time PCR was performed using Fast SYBR Green Master Mix (Thermo Fisher Scientific). Data were analyzed as the ratio to GAPDH.

Significance Statement
Branching morphogenesis of the ureteric bud is central to forming a normal kidney, and the most severe forms of congenital anomalies of the kidney and urinary tract (CAKUT) arise from mutations in genes involved in branching. The authors report that deletion in mice of transcription factor 21 (Tcf21) results in a spectrum of renal developmental phenotypes that resemble human CAKUT. Germline deletion of Tcf21 or Tcf21 deleted specifically from the stromal mesenchyme resulted in branching defects and reduced expression of Gdnf-Ret-Wnt11 (a key pathway required for branching morphogenesis), whereas Tcf21 deletion specifically from the cap mesenchyme resulted in glomerular rather than branching defects and no downregulation of Gdnf-Ret-Wnt11. These findings suggest that Tcf21 has a central role in regulating the Gdnf axis and renal stromal factors crucial for branching.

Immunostaining
For immunohistochemistry and lectin stainings, kidneys were dissected and fixed by 10% formalin or 4% paraformaldehyde overnight. Tissues were then embedded in paraffin and sliced into 5-µm-thick sections. Before immunostainings, sections were deparaffinized in xylene and ethanol and boiled in citrate buffer for antigen retrieval. For immunofluorescence, after fixing by 4% paraformaldehyde overnight, tissues were cryoprotected in 30% sucrose overnight and embedded in Tissue-Tek O.C.T 4583 compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) at −80°C and sliced into 10-µm-thick sections.

These sections were blocked (0.3% Triton X-100, 3% albumin, 1 mM MgCl2, 1 mM CaCl2 in PBS) for 1 hour, incubated with primary antibodies at 4°C overnight. After washing several times by PBS, they were incubated with secondary antibodies for 1 hour at room temperature. Fluorescence-labeled Dolichos biflorus agglutinin (DBA) or Lotus tetragonolobus lectin (LTL) was mixed with secondary antibodies. For detailed methods and a full list of antibodies, see Supplemental Material and Supplemental Table 1.
Figure 1. UB branching is impaired and Gdnf, Ret, and Wnt11 are downregulated in Tcf21 null kidneys. (A and B) Reduced number of UB tips (green, calbindin) and renal vesicles (red, Jagged1) in Tcf21 null kidney explants. Kidneys were dissected at E12.5, incubated for 48 hours and subjected to immunostainings. Scale bar, 50 μm. (C) Downregulation of Gdnf, Ret, and Wnt11 mRNA expression in Tcf21 null kidneys at E14.5 by quantitative RT-PCR. (D) Low expression of Gdnf mRNA in the mesenchyme of Tcf21 null kidneys at E12.5 and E14.5 by in situ hybridization. Scale bar E12.5, 100 μm; E14.5, 50 μm. (E) Paucity of Ret and Wnt11 mRNA at the UB tips by in situ hybridization in Tcf21 null kidneys at E14.5. Scale bar, 50 μm. (F) Immunostaining of Gdnf and the pan-epithelial marker E-cadherin of Tcf21 null kidneys and controls at E14.5 and E16.5 showing low expression of Gdnf and abnormal UB branching. Scale bar, 100 μm. *P<0.05; **P<0.01; ***P<0.001.
Explant Culture of Mouse Embryonic Kidneys
Embryos were dissected from pregnant mice on day 12.5 postcoitum. Kidneys from embryos were cultured on Nucleopore Track-Etched Membranes (Whatman) floated on culture medium. After 48 hours, kidneys were fixed in 4% PFA and stained for Calbindin and Jagged1. The number of branching and Jagged1-positive spots were counted in a double-blind manner.

mRNA In Situ Hybridization
mRNA in Situ hybridization was performed on formalin-fixed, paraffin-embedded sections using the RNAscope 2.5 assay system (Advanced Cell Diagnostics) with RNAscope FFPE Reagent Kit, 2.5 HD-Reagent Kit-RED, 2.5 HD-Reagent Kit-BROWN. Recommended probes were used for this assay.

Statistical Analyses
Statistical analyses were carried out using GraphPad Prism 6.0 (GraphPad Software Inc.). Comparison of two groups was done by two-tailed t-test. A P-value <0.05 was considered significant. Error bars showed ±SEM.

RESULTS

Tcf21 Regulates Branching Morphogenesis via Gdnf-Ret-Wnt11 Signaling
Germline deletion of Tcf21 leads to hypodysplastic kidneys reminiscent of CAKUT in humans (Supplemental Figure 1). At E12.5 +48 hours, Tcf21 null explant cultures show a very abnormal UB tree (Figure 1, A and B). To determine how Tcf21 regulates UB branching and collecting duct development, we first examined the expression of Gdnf, which is critical for branching morphogenesis. Gdnf transcript level was markedly downregulated to <40% in Tcf21 null kidneys at E14.5 compared with wild-type by quantitative RT-PCR (Figure 1C). By in situ hybridization at E12.5 and E14.5 and by immunohistochemistry at E14.5 and E16.5, Tcf21 null kidneys also exhibit reduction of Gdnf (Figure 1, D and F). Next, we examined the expression of Ret, the cognate tyrosine kinase receptor for Gdnf, and that of Wnt11, a growth factor that is necessary to maintain Gdnf expression. Similar to Gdnf, both Ret and Wnt11 transcript levels were decreased in Tcf21 null kidneys at E14.5, consistent with paucity of UB branch tips (Figure 1, C and E). The quantitative RT-PCR results were normalized to Gapdh to account for size difference of the kidneys. These results suggest that Tcf21 is required for normal expression of Gdnf-Ret-Wnt11 and therefore is critical for UB branching. On the other hand, the expression pattern of markers for CM (Six2, Pax2, and Wt1) was not decreased by quantitative RT-PCR and immunostaining in Tcf21 null kidneys (Figure 2, A and B). This suggests that the decrease of Gdnf in Tcf21 null kidneys is not the result of loss of nephron progenitor population, consistent with previous experiments.27 Further, the transcription factors that regulate Gdnf (Osr1, Eya1, Pax2, Six2, Hoxa11, Hoxd11, Wt1, Sall1, and Gata3) were not decreased in Tcf21 null kidneys (Figure 2, Supplemental Figure 2). We next examined the potential role of Tcf21 in non-Gdnf-dependent pathways that regulate UB branching: fibroblast growth factor (Fgf), canonical Wnt, and retinoic acid signaling. At E14.5 and E18.5, none of the transcripts of Aldehyde dehydrogenase family 1, subfamily a2 (Aldh1a2), Retinoic acid receptors (Rara), Fgf7, Fgf10, Retinoic acid receptor β (Rarb), and Wnt4 were significantly downregulated (Supplemental Figure 3). Taken together,
these results support a selective role for Tcf21 in regulating the Gdnf axis.

**Tcf21 Has Distinct Roles in the Stromal and CM during Kidney Development**

When MM cells begin to aggregate around the UB tips, the mesenchyme segregates to CM (expressing Cited1 and Six2) and to stromal mesenchyme (expressing high levels of Foxd1). We evaluated Tcf21 gene expression in each cell compartment using Tcf21^{LacZ/Wild}, Foxd1-eGFPCre; ROSA26-mTmG reporter mice. Immunostaining demonstrated that Tcf21 is distributed both in a subset of the Six2-positive CM and in the derivatives of Foxd1-positive stromal mesenchyme (Figure 3A). We hypothesized that the defect observed in UB branching and Mesenchymal to epithelial transformation in Tcf21 null kidneys represented convergence of signals from these distinct cell lineages that arise from the MM. To dissociate these effects, we generated two Cre/LoxP mouse models, StrTcf21 and CapTcf21, where Tcf21 is selectively deleted from the kidney stromal cells under

**Figure 3.** Tcf21 is expressed in a subset of the CM and stromal mesenchyme. (A) Immunostaining of the kidney from Tcf21^{LacZ/+}; FoxD1Cre; (ROSA26)mT/mG triple transgenic mice. Tcf21 LacZ expression by β-galactosidase staining (red) delineating normal expression of Tcf21 at E18.5. Derivatives of FoxD1-positive stromal mesenchyme was visualized by GFP using FoxD1-Cre; (ROSA26)mT/mG reporter mice (green). CM is represented by Six2 immunostaining (blue). Bar, 100 μm. (B) Schematic depiction of Tcf21 deletion in the mouse models utilized. White areas represent Tcf21-deleted cell lineages. (i) Wild-type; (ii) Tcf21 null kidney, Tcf21 is deleted in germline cells and hence from the entire MM and its derivatives; (iii) CapTcf21 kidney, Tcf21 is selectively deleted from the CM under Six2-eGFPCre driver; (iv) StrTcf21 kidney, Tcf21 is selectively deleted from the stromal mesenchyme under Foxd1-eGFPCre driver.
**BASIC RESEARCH**

**Deletion of Tcf21 in Stromal Progenitors Results in Abnormal UB Branching, Paucity of Collecting Ducts, and Disorganized Stroma**

Several lines of evidence support a role for Tcf21 in the development of the interstitial stroma. To study Tcf21’s function in the stroma, Tcf21**−/−** mice were bred to *Foxd1-eGFPCre* driver mice to generate a stromal-specific Tcf21 knockout mice (StrTcf21). In this model, Tcf21 mRNA expression in the stroma is markedly decreased starting at E14.5 onwards, although it is still expressed at E12.5 (Supplemental Figure 4). When the StrTcf21 mice were evaluated, they appeared healthy and survived for at least 10 weeks. On gross dissection, the StrTcf21 kidneys are reduced in size at birth and their histology reveals normal glomeruli but disorganized stroma (Figure 4, A and B, Supplemental Figure 5A). Notably, at 4 weeks of age the StrTcf21 animals showed increased urine volume and a severe defect in urine concentration capacity (Figure 4C). Corresponding with this finding is the severe reduction of collecting ducts in StrTcf21 kidneys that markedly outnumber the mild reduction in proximal tubules (Figure 4, D and E, Supplemental Figure 5, B and C). The paucity of collecting ducts is also evident by reduction of Aquaporin 2, Calbindin, DBA, *Epithelial sodium channel*, and *Na–H-ATPase* (Figure 4D, Supplemental Figure 5, C and D). To investigate whether the loss of collecting ducts stemmed from abnormal UB development, we examined the UB tree in kidney explants and noticed fewer UB tips in the StrTcf21 kidneys at E12.5 +48 hours, suggesting abnormal branching although we cannot rule out the possibility that artifacts from culturing for 48 hours may be different in mutants versus controls. Additionally, *Wnt7b* was decreased at E14.5 (Figures 4, F and G and 5C). *Wnt7b* is a UB stalk maker important to corticomedullary organization and function. Further supportive of a primary branching defect is the finding of low expression of *Gdnf* and *Wnt11* mRNA in the StrTcf21 kidney at E14.5 and P0 (Figure 5, A and B, Supplemental Figure 5, E and F). Of note, although Ret mRNA level is not reduced here, its downstream effectors *Etv4* and *Etv5* are significantly downregulated at E14.5 and E18.5 in StrTcf21 kidneys (Figure 5A, Supplemental Figure 5E). Taken together, these results suggest that *Tcf21* expression in stromal cells is required for normal expression of the Gdnf axis, development of the collecting ducts, and normal organization of the stroma, whereas expression of members of the retinoic acid and Fgf pathways do not appear to be Tcf21-dependent (Figure 5, D and E). Although the stroma of StrTcf21 mice appears disorganized, mRNA levels of stromal markers were not depressed in whole-kidney lysate from mutants at E14.5 and P0 supporting normal stromal cell mass (Supplemental Figure 6).

**Tcf21 in the CM Is Necessary for Proper Development of the Glomerulus and Tubules But Not for the Collecting Ducts**

Deletion of *Tcf21* from the CM (CapTcf21) results in smaller kidneys (Figure 6A, Supplemental Figure 7A) and decreased urine volume at P0 and proteinuria at 3 days of age (Figure 6B). Histologically at P0, the glomeruli of CapTcf21 show primitive and simplified structures. The tubules are cystic and atrophic, but the stromal structure does not show obvious changes (Figure 6C). The simplified glomeruli show decreased Podocin and Desmin, suggestive of a defect in mesangial ingrowth and/or mesangiolysis (Figure 6D), and ultrastructurally, the podocytes have effaced foot processes (Supplemental Figure 7B). The loss of tubular mass is also shown by reduced area of LTL-expressing proximal tubules and Uromodulin-positive thick ascending limb (Figure 6E). These results indicate that loss of *Tcf21* in Six2-positive progenitors leads to defects in glomerulogenesis, podocyte differentiation, and tubular development. In contrast, absence of *Tcf21* does not obviously affect the distribution of the collecting ducts as shown by similar DBA-positive area between CapTcf21 and controls (Figure 6F). In line with these findings are the results from kidney explants that show reduction in both renal vesci cles (Jagged1) and proximal tubules (LTL), but only a mild decrease in UB tip number in CapTcf21 explants (Figure 7, A and B). The minimal effect of Tcf21 on branching in CapTcf21 kidneys is also noted by E-cadherin staining at E14.5 and similar mRNA levels of UB markers Wnt7b and Sox9 (Figure 7C, Supplemental Figure 7C). Importantly, loss of *Tcf21* in the CM did not attenuate expression of *Gdnf*, *Ret*, or *Wnt11* transcripts at E14.5 (Figure 7D). Taken together, these results suggest that although *Tcf21* in the CM is required for the normal development of the glomeruli and tubules, it is not necessary for normal Gdnf axis and development of the collecting ducts.

**Bmp4 Is Upregulated in Tcf21 Null Kidneys**

As described above, decreased Gdnf levels were noted only in *Tcf21* null and StrTcf21 kidneys, whereas Gdnf had normal

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**Figure 4.** Absence of Tcf21 from the renal stroma does not affect glomerulogenesis but results in urine concentration defect, abnormal collecting ducts, and branching morphogenesis. (A and B) StrTcf21 kidneys are reduced in size, have normal glomeruli, and disorganized stroma (B) Scale bars: top, 500 μm; middle, 25 μm; bottom, 50 μm. (C) Strtcf21 mice develop polyuria and urine concentration defect at 4 weeks. (D and E) StrTcf21 kidneys show severe decrease of inner medulla. Thick ascending limbs that express Uromodulin and collecting ducts that express Aquaporin 2 are largely reduced. Scale bar, 500 μm. (F and G) Abnormal UB branching in kidney explants of StrTcf21 dissected at E12.5. Scale bar, 50 μm. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.
Figure 5. Absence of Tcf21 from the renal stroma leads to downregulation of the Gdnf axis at E14.5. (A and B) StrTcf21 kidneys have low mRNA of Gdnf and Wnt11 on quantitative real-time PCR and in situ hybridization. Ret transcript is not reduced but Etv4 and Etv5, downstream effectors of Ret, are downregulated in this model. Scale bar, 50 µm. (C) Reduced expression of the UB stalk marker Wnt7b, shown by quantitative real-time PCR. Expression of the pan-epithelial marker E-cadherin is not decreased. (D and E) mRNA expression of retinoic acid and Fgf signaling components that are critical for UB branching are not significantly changed by in situ hybridization and quantitative real-time PCR. Scale bar, 100 µm. *P<0.05; **P<0.01.
Figure 6. Absence of Tcf21 from the CM leads to abnormal glomeruli and tubules but does not affect the collecting ducts. (A) Absence of Tcf21 from the CM results in smaller kidneys at P0. (B) Urine volume is reduced and proteinuria is evident at P0 and P3 in CapTcf21 mice (volume measured by aspirating bladder content at the time of dissection). (C) Periodic acid–Schiff staining shows small and simplified glomeruli and cystic tubules in CapTcf21 mice. The histology of the renal stroma is not different from controls.
expression in the CapTcf21 model, suggesting that branching is dependent on Tcf21 expression in the stroma. Bmp4 is a member of the TGF-β superfamily that is expressed throughout embryonic development, initially in stromal cells enveloping the nephric duct.30–35 BMP4 is one of the key regulators of UB branching, inhibiting ectopic UB outgrowth by decreasing expression of Gdnf.34,36–38 Previous work showed that Tcf21-lacZ-expressing cells are present around the stalk of the UB and express Bmp4.27 Here, Bmp4 protein and mRNA levels were increased in Tcf21 null kidneys at E18.5 (Figure 8, A and B). Upregulation of Bmp4 was also seen in our previously conducted gene-expression microarray analysis on total RNA extract from glomeruli of Tcf21 lacZ/lacZ null mice at E18.5 (Mouse Affymetrix GeneChips 430–2.0)39 (Figure 8C). In silico analysis showed that the 3000 bp promoter and 5’ UTR regions of the Bmp4 gene contain the canonical binding sequences of Tcf21.40 (Figure 8D). Thus, Bmp4 expression increase in Tcf21 null kidneys may suggest its involvement in Tcf21-mediated effects.

**DISCUSSION**

In the mammalian kidney, interactions between the UB and the MM promote growth and differentiation to generate a functional organ of appropriate size, shape, and cellular diversity.31 Perturbations to these processes lead to CAKUT that, in many cases, stem from disruption of genes involved in renal organogenesis.42,43 The “budding theory” proposes that hypodysplastic kidneys in CAKUT are the result of defects in UB branching.44,45 UB epithelium branching is principally controlled by the Gdnf-Ret pathway and by the Wnt, Fgf, and retinoic acid signaling pathways, but many other genes are involved.2,46–48 Importantly, mutations in RET and GDNF, as well as in other genes that regulate branching of the UB, have been associated with CAKUT.10,42 Although a functional role of Tcf21 in human CAKUT has not yet been reported, Tcf21 has been shown to be downregulated in dysplastic fetal kidneys.49

Here, we report a spectrum of renal developmental phenotypes that resemble human CAKUT that result from loss of Tcf21 from the germline, or from specific MM compartments. Kidneys from conventional Tcf21 knockout mice show arrested Mesenchymal to epithelial transformation, with malformed and very small kidneys. We also identified a reduction in expression of the Gdnf-Ret-Wnt11 signaling loop critically required for branching morphogenesis in null Tcf21 embryos.

The expression of Tcf21 in the developing kidney is complex.27 Although Tcf21 is not expressed in metanephrogenic blast cells found at the periphery of the metanephros before induction and condensation, it is expressed in both the Six2-expressing CM and in FoxD1-expressing stromal cells. Somewhat unexpectedly, the branching defects observed in conventional Tcf21 knockout mice were only observed in mice lacking Tcf21 from the stromal compartment, whereas glomerular defects were observed in CapTcf21 mutants. In addition, Gdnf-Ret-Wnt11 downregulation was only observed in StrTcf21 and total knockout mice.

What can be learned from these distinct phenotypes? The most likely explanation is that Tcf21 has distinct functions in the stromal versus cap mesenchymal lineages. Interestingly, the phenotype of the Tcf21 null mice was much more severe than that observed in either StrTcf21 or CapTcf21 kidneys, suggesting that the Tcf21 null phenotype is not a simple sum of each compartmentalized phenotype. Although this may reflect timing or completeness of gene inactivation, other explanations are possible, including crosstalk between Tcf21-expressing cell types or existence of additional subset(s) of mesenchymal cell types not represented by Six2 or FoxD1 expression.

The role of the stromal cells as a key regulator of nephrogenesis and branching morphogenesis has previously been shown in Foxd1 null mice.50–52 Levinson et al.51 showed that Foxd1 null mice are born with hypodysplastic and fused midline kidneys, a similar but not identical renal phenotype observed in Tcf21 null mice. The importance of stromal Tcf21 for UB branching has been previously suggested in Tcf21lacZ/GFP chimeras, alluding to a non-cell-autonomous role of Tcf21 in the stroma.27 We now have shown that removal of Tcf21 in Foxd1-positive cells leads to downregulation of Gdnf-Ret-Wnt11, providing a potential mechanism to explain the phenotypes of both Tcf21 and Foxd1 knockout mice. Whether Tcf21 expression in the stroma is required for normal stromal development is not completely clear. Here, we show that removal of Tcf21 from stromal progenitors results in disorganized interstitium that has normal expression of stromal cell markers, suggesting that Tcf21 is not required for the development of normal total stromal cell mass but that it may have a role in stromal cell alignment. Lastly, identifying direct gene targets for Tcf21 in the kidney is crucial for understanding its mechanistic action. In that regard, the observed increased levels of Bmp4, a stromal factor and a potent inhibitor of UB branching and Gdnf,33,34,53–56 is

Scale bar, 50 μm. (D) CapTcf21 kidneys demonstrate glomerular injury by reduced Podocin and Desmin. Scale bars, 25 μm. (E) Reduced area of proximal tubules (LTL) and thick ascending limb (Uromodulin) in CapTcf21 kidneys. Scale bar, 500 μm. (F) The collecting ducts (DBA) are thick but the area is similar in CapTcf21 compared with controls. Scale bar, 500 μm. *P<0.05; **P<0.01; ***P<0.001; ****P<0.001.
intriguing; however, further study is required to verify direct regulatory effect.

In summary, the results support a model whereby Tcf21 has distinct roles in cap versus stromal MM that are critical for normal nephrogenesis. In addition, stromal expression of Tcf21 is needed to allow normal UB branching morphogenesis via the Gdnf-Ret-Wnt11 autoregulatory loop (Supplemental Figure 8), underscoring...
the importance of stromal-cap-MM-UB crosstalk in kidney development.

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DISCLOSURES

S.E.Q. owns stock in and is a director of Mannin Research, and is on the external advisory board of Astra Zeneca.
REFERENCES


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**AFFILIATIONS**

1Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan; 2Division of Kidney Diseases, Ann and Robert H. Lurie Children’s Hospital of Chicago, Chicago, Illinois; 3Feinberg Cardiovascular and Renal Research Institute and 4Division of Nephrology/Hypertension, Northwestern University Feinberg School of Medicine, Chicago, Illinois; 5Department of Anatomy, Kyorin University School of Medicine, Tokyo, Japan; 6Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; and 7Division of Diabetes, Metabolism and Endocrinology, International University of Health and Welfare, Narita, Japan