Galnt11 regulates kidney function by glycosylating the endocytosis receptor megalin to modulate ligand binding

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Chronic kidney disease (CKD) affects more than 20 million Americans and ∼10% of the population worldwide. Genome-wide association studies (GWAS) of kidney functional decline have identified genes associated with CKD, but the precise mechanisms by which they influence kidney function remained largely unexplored. Here, we examine the role of 1 GWAS-identified gene by creating mice deficient for *Galnt11*, which encodes a member of the enzyme family that initiates protein O-glycosylation, an essential posttranslational modification known to influence protein function and stability. We find that *Galnt11*-deficient mice display low-molecular-weight proteinuria and have specific defects in proximal tubule-mediated resorption of vitamin D binding protein, α_1-microglobulin, and retinol binding protein. Moreover, we identify the endocytic receptor megalin (LRP2) as a direct target of Galnt11 in vivo. Megalin in *Galnt11*-deficient mice displays reduced ligand binding and undergoes age-related loss within the kidney. Differential mass spectrometry revealed specific sites of Galnt11-mediated glycosylation within mouse kidney megalin/LRP2 that are known to be involved in ligand binding, suggesting that O-glycosylation directly influences the ability to bind ligands. In support of this, recombinant megalin containing these sites displayed reduced albumin binding in cells deficient for *Galnt11*. Our results provide insight into the association between *GALNT11* and CKD, and identify a role for Galnt11 in proper kidney function.

megalin | O-glycosylation | kidney disease | proteinuria | GALNT11

Chronic kidney disease (CKD) affects more than 10% of adults across the globe, and thus is a major public health concern (1, 2). However, many factors contributing to CKD remain largely unknown, hampering our ability to design effective treatments. Recent genome-wide association studies (GWAS) have identified genes associated with CKD, but a functional role for many remains to be validated (3–5). Indeed, 1 study investigating genetic loci associated with a decline in renal function over time identified a single nucleotide polymorphism located in the intron of an inactive member of the *GALNT* family (*GALNTL5*, now known as *GALNT20*) in linkage disequilibrium with *GALNT11*, *MLL3*, and *CCT8L1* (4). Morpholino knockdown of the *GALNT11* and *MLL3* orthologs in zebrafish did not result in overt kidney defects, but did make kidneys more susceptible to nephrotic insults (4). However, whether any of the genes within this genetic locus play a direct role in kidney function remains unknown.

GALNT11 (*Galnt11* in mice) encodes a member of the large glycosyltransferase family responsible for initiating mucin-type O-glycosylation of secreted and membrane-bound proteins (6, 7). The ortholog of *Galnt11* was first characterized in *Drosophila* and shown to be essential for viability (8, 9) and involved in tubulogenesis (10). More recently, cell culture studies have demonstrated that Galnt11 glycosylates members of the low-density lipoprotein receptor (LDLR) family in the linker sequence between LDLR class A (LA) repeats (11) and enhances ligand binding (12). Members of the Galnt family (20 members in mammals and 10 in *Drosophila*) display unique expression patterns and substrates specificities (6, 7, 13–16). Moreover, studies in model systems have demonstrated roles in diverse biological processes including protease processing, secretion, cell signaling, cell adhesion, and organ growth (7, 17–24).

Here, we directly examine the role of *Galnt11* in mammalian kidney function by creating mice deficient for *Galnt11*. We find that *Galnt11*-deficient mice suffer from low-molecular-weight proteinuria. Moreover, we identify the endocytic receptor megalin/LRP2, a member of the LDLR family, as a specific in vivo substrate of Galnt11 in the kidney. In the absence of Galnt11, megalin shows reduced binding to endogenous ligands and undergoes an age-related decline in abundance, suggesting that O-glycosylation of this region modulates ligand binding in vivo. In support of this, recombinant megalin containing these sites of differential glycosylation displayed reduced ligand binding in cell

Significance

Chronic kidney disease (CKD) remains a major health concern worldwide, affecting ∼10% of the population. Previous genome-wide association studies have identified many genes associated with CKD, but conclusive demonstrations of their roles in kidney function remain largely unexplored. Here, we identify the mechanistic basis of one such gene by creating mice deficient for *Galnt11*. We demonstrate that Galnt11 influences kidney function by site-specific modification of the endocytosis receptor megalin within the proximal tubules of the kidney. Loss of *Galnt11* results in reduced megalin-mediated ligand binding and an age-related reduction in megalin levels, resulting in low-molecular-weight proteinuria. Our results identify a factor required for proper kidney function and provide insight into its association with CKD.

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The authors declare no competing interest.

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Data deposition: Mass spectrometry data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (Galnt11 differential tissue glycoproteomics, accession no. PXD015429).

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lines deficient for *GALNT11*. Our results provide mechanistic insight into the association between *GALNT11* and CKD, as well as the role of Galnt11 in proper kidney function.

Results

Loss of *Galnt11* Results in Low-Molecular-Weight Proteinuria. Previous GWAS identified *GALNT11*, a gene encoding a conserved GalNAc-transferase responsible for initiating O-linked glycosylation (*SI Appendix*, Fig. S1*A*), as being associated with kidney functional decline (4). Indeed, *Galnt11* is the most abundant *Galnt* family member expressed in the mouse kidney (Fig. 1*A*), and is specifically expressed in the proximal tubules, as determined by in situ hybridization (Fig. 1*B*), similar to expression patterns seen in human kidneys (8). We therefore set out to examine its effects on kidney function by creating mice deficient for *Galnt11* (*SI Appendix*, Fig. S1 *B* and *C*). Mice homozygous deficient for *Galnt11* ($Galnt11^{-/-}$) displayed reduced size relative to wild-type (*WT*; $Galnt11^{+/+}$) and heterozygous ($Galnt11^{+/-}$) littermate controls and reduced viability over time (Fig. 1 *C* and *D*). Kidney histology and complete blood count analysis did not reveal differences among *WT*, heterozygous ($Galnt11^{+/-}$), and $Galnt11^{-/-}$ animals (*SI Appendix*, Fig. S2 *A* and *B*). No significant differences among *WT*, $Galnt11^{+/-}$, and $Galnt11^{-/-}$ were observed in blood or urine creatinine levels (*SI Appendix*, Fig. S2 *C* and *D*). However, a significant increase in blood urea nitrogen was seen in $Galnt11^{-/-}$ animals (*SI Appendix*, Fig. S2*E*), suggesting an effect on kidney function. Notable decreases in staining indicative of proteins decorated with O-linked glycans (as detected by the lectin peanut agglutinin or PNA) were seen in the cortex of $Galnt11^{-/-}$ kidneys relative to *WT* (*SI Appendix*, Fig. S3*A*), whereas decreases in PNA staining were not obvious in tissues where *Galnt11* transcripts are less abundant relative to other family members (liver and lung; *SI Appendix*, Fig. S3 *A*–*C*). Interestingly, $Galnt11^{-/-}$ mice also displayed increased albumin-to-creatinine ratios relative to *WT* littermate controls, suggesting that the loss of *Galnt11* affects the ability of kidneys to resorb albumin (Fig. 1*E*). In addition, increased levels of low-molecular-weight proteins, including vitamin D binding protein (DBP), α1-microglobulin (α_1-M), and retinol binding protein (RBP), were seen in the urine of $Galnt11^{-/-}$ animals relative to *WT* controls (Fig. 1*F*). These results indicate that the loss

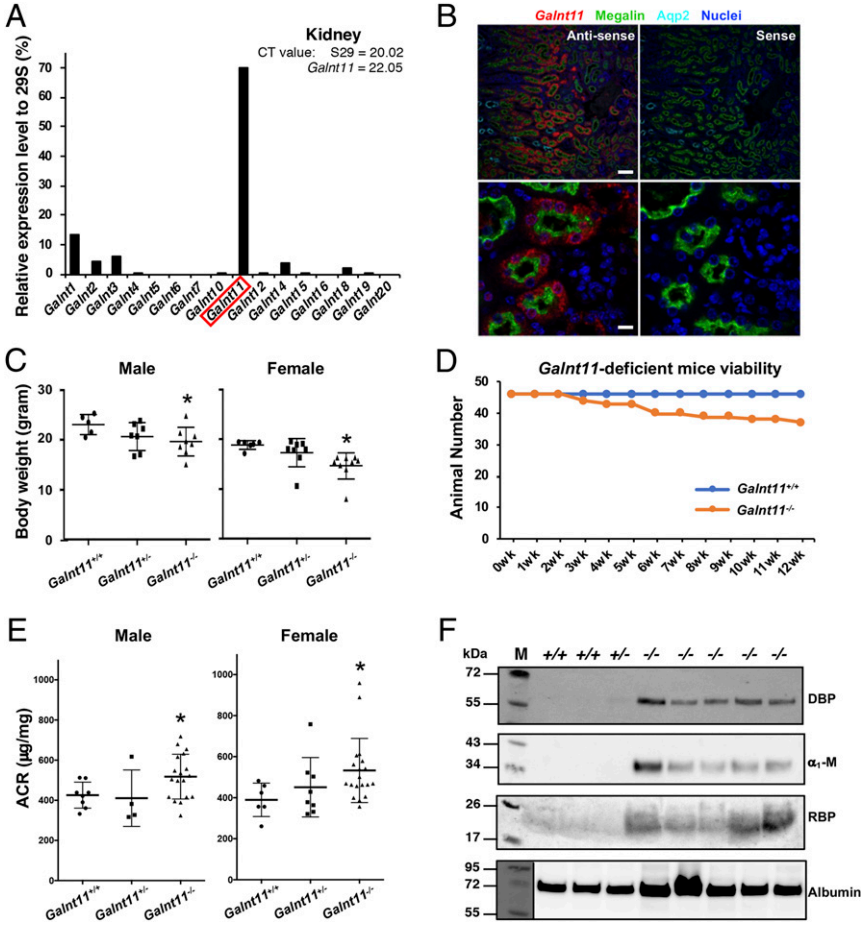

Fig. 1. Loss of *Galnt11* results in decreased viability and low-molecular-weight proteinuria. (*A*) *Galnt11* is the most abundant family member expressed in the kidney as determined by qPCR. Expression levels were normalized to *29S* for each *Galnt* family member. (*B*) *Galnt11* is abundantly expressed in the proximal tubules of the kidney. In situ hybridization shows that *Galnt11* mRNA (red; anti-sense) is expressed specifically in the proximal tubules of the adult kidney, as detected by costaining with the proximal tubule marker megalin (green). No *Galnt11* expression was seen in the distal tubules, which are stained with aquaporin-2 (cyan). Nuclear staining is shown in blue. The *Galnt11* sense control probe is shown in the *Right* column. (Scale bar, 100 μm [*Upper*] and 10 μm [*Lower*].) (*C*) $Galnt11^{-/-}$ mice have lower body weight than heterozygous ($Galnt11^{+/-}$) and *WT* ($Galnt11^{+/+}$) littermate controls. (*D*) $Galnt11^{-/-}$ mice show reduced viability as they age relative to *WT* controls. (*E*) $Galnt11^{-/-}$ mice display elevated urine albumin/creatinine ratios (ACRs) relative to *WT* ($Galnt11^{+/+}$) and heterozygous ($Galnt11^{+/-}$) littermate controls. ACRs are shown for individual animals, with males on the left and females on the right. Center bar is the mean. $*P < 0.05$. (*F*) $Galnt11^{-/-}$ mice have low molecular weight proteinuria relative to *WT* and heterozygous ($Galnt11^{+/-}$) littermate controls. Western blots were probed with antibodies to vitamin D binding protein (DBP), α1-microglobulin (α_1-M), retinol binding protein (RBP) and albumin. All lanes in each blot are contiguous but the vertical black line in the bottom panel denotes different exposures of marker and sample lanes.

of *Galnt11* affects growth, viability, and low-molecular-weight protein reabsorption.

Previous cell culture studies have shown the Galnt11 can glycosylate a linker sequence in the LA binding modules of LRPs and influence ligand binding of LDLR and VLDLR (12). The endocytic receptor megalin (LRP2) is a member of the LDLR family expressed in the proximal tubules of the kidney, where it is responsible for binding and endocytosing albumin and other low-molecular-weight proteins (DBP, α_1-M, and RBP) (25–27). Therefore, we next examined megalin in *Galnt11*$^{-/-}$ animals. As shown in Fig. 2*A*, megalin is normally present along the apical surface of the kidney proximal tubules and overlaps with PNA staining. However, in *Galnt11*$^{-/-}$ animals, PNA-reactive O-glycans are not seen along the apical surface, and megalin staining is reduced (Fig. 2*A*). These results were seen with and without neuraminidase treatment. To determine when megalin levels are first altered along the proximal tubules, we next compared megalin abundance in *Galnt11*$^{-/-}$ animals relative to *WT* littermate controls at various ages, beginning at embryonic day 17 (E17) and continuing through adulthood (6 to 12 wk; Fig. 2 *B–D*). Confocal imaging revealed that megalin is present along the apical surface of the proximal tubules in *Galnt11*$^{-/-}$ and *WT* littermate controls

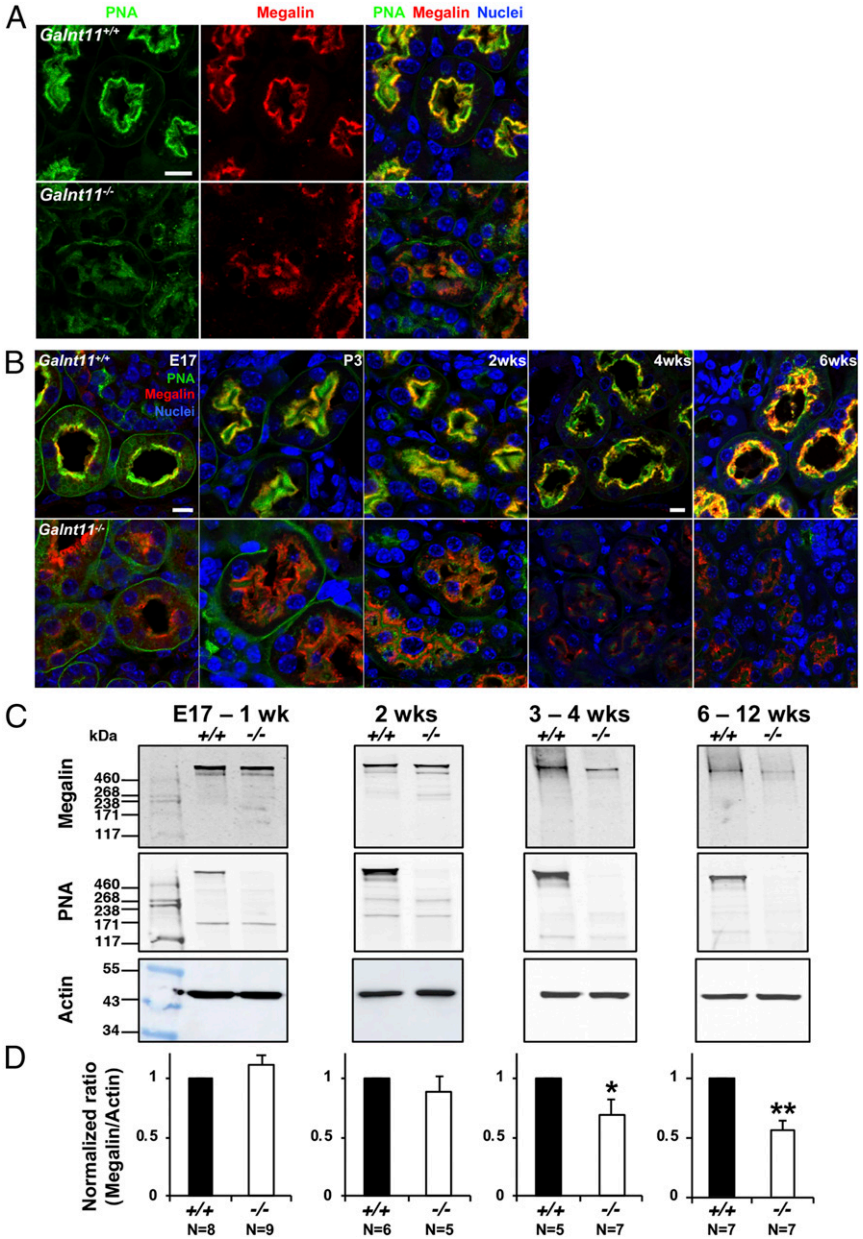

Fig. 2. Megalin levels undergo an age-related decrease in *Galnt11*$^{-/-}$ kidneys. (*A*) Immunofluorescence reveals a specific decrease in O-glycans (as detected by the lectin PNA, green) and megalin (red) along the apical surface of the proximal tubules in the *Galnt11*$^{-/-}$ kidneys relative to *WT* littermate controls at 3 wk of age. Nuclear staining is shown in blue. (Scale bar, 10 μm.) (*B*) An age-related decrease in megalin is seen along the apical surface of *Galnt11*$^{-/-}$ proximal tubules. Ages are shown in the *Upper Right* corner of the *Upper* panels. (Scale bar, 10 μm.) (*C*) Western blots of kidney extracts from *WT* and *Galnt11*$^{-/-}$ animals at various ages (E17-1 wk; 2 wk; 3 to 4 wk; 6 to 12 wk) show that megalin levels decrease in an age-related manner in the absence of *Galnt11*. Additionally, the major O-glycan band (detected by the lectin PNA) that overlaps in position with megalin is absent in *Galnt11*$^{-/-}$ animals of all ages. Actin loading controls are shown in the *Lower* panels. (*D*) Ratios of megalin/actin in mice of each age group. *n* = the number of mice analyzed in each group. Average ratio of *WT* was set to 1 in each age group. $*P < 0.05$; $**P < 0.01$.

at E17 until ∼4 wk of age. At 4 wk, megalin along the apical surface begins to decrease in abundance in *Galnt11*$^{-/-}$ animals relative to *WT* (Fig. 2*B*). Western blots of cortical kidney extracts also demonstrated an age-related decline in total megalin abundance (normalized to actin) beginning at 4 wk in *Galnt11*$^{-/-}$ animals (Fig. 2 *C* and *D*). No change in the levels of cubilin (the other major endocytic receptor in the proximal tubules) at any age in *Galnt11*$^{-/-}$ versus *WT* (*SI Appendix*, Fig. S4) were seen, suggesting that the *Galnt11* effects are specific to megalin. Western blots also revealed the loss of a specific O-glycosylated protein (PNA-reactive band) corresponding in size to megalin in the *Galnt11*$^{-/-}$ animals, suggesting that megalin may be the direct target of Galnt11-mediated glycosylation in WT kidneys (Fig. 2*C*). These results suggest that the loss of *Galnt11* may be specifically affecting megalin stability and/or function in adult kidneys.

We also examined cholesterol levels in *Galnt11*$^{-/-}$ mice, given previous cell culture studies implicating Galnt11-mediated glycosylation in the ability of LDLR to bind to LDL (12). As shown in *SI Appendix*, Fig. S3*B*, *Galnt11* is 1 of 11 *Galnt* family members expressed in mouse liver. *Galnt11*$^{-/-}$ and *WT* control mice were fed a high-fat Western diet, and at 11 to 12 wk of age, cholesterol levels were measured. There was no significant change in total cholesterol or triglyceride levels when comparing individual *Galnt11*$^{-/-}$ with *WT* mice (*SI Appendix*, Fig. S5 *A* and *B*). However, when samples were pooled and plasma lipoproteins fractionated by FPLC, an ∼2-fold increase in LDL-cholesterol in female *Galnt11*$^{-/-}$ mice and an ∼1.5-fold increase in LDL-cholesterol in male *Galnt11*$^{-/-}$ mice relative to *WT* was observed (*SI Appendix*, Fig. S5 *C* and *D*), suggesting that the loss of *Galnt11* may influence LDLR function in vivo.

Galnt11 Glycosylates Megalin and Affects Ligand Binding. We next set out to determine whether megalin along the apical surface of the proximal tubules in *Galnt11*$^{-/-}$ animals before 4 wk of age (a time when megalin is equally abundant along the apical surface in both *WT* and *Galnt11*$^{-/-}$ animals) is capable of binding ligands. Western blots of urine collected from *Galnt11*$^{-/-}$ and *WT* littermate controls at 2, 3, and 4 wk of age were probed to detect the presence of ligands that are normally bound by megalin and recovered from the filtrate (and therefore should not be present in the urine). As shown in Fig. 3*A*, megalin ligands (DBP and α_1-M) are found in the urine of *Galnt11*$^{-/-}$ animals, whereas they are not present in the urine of *WT* controls. To quantify the amount of ligands bound relative to megalin present, we next stained kidney sections from 2-wk-old *Galnt11*$^{-/-}$ and *WT* littermate controls with megalin and a megalin ligand (DBP). As shown in Fig. 3 *B*–*D*, a dramatic reduction in the amount of DBP concentrated at the apical surface of the proximal tubules (normalized relative to the amount of megalin present) was seen in the absence of *Galnt11*. Taken together, these results suggest that the loss of *Galnt11* is affecting the ability of megalin to bind ligands.

We next set out to determine whether megalin is a direct target of Galnt11 in vivo. Megalin immunoprecipitated from *WT* mouse kidney extracts reacts with the lectin PNA, indicating that megalin is normally O-glycosylated (Fig. 4*A*). To further explore the functional effect of Galnt11 in murine kidneys, we used a quantitative O-glycoproteomics strategy described previously (28). Differential quantitation of O-glycopeptides from kidney tissue extracts confirmed the glycosylation status of megalin in *WT* animals and further revealed specific amino acid residues that are underglycosylated or no longer glycosylated in the absence of *Galnt11* (Fig. 4*B* and *SI Appendix*, Table S1) (29). In total, we identified 13 glycosylation sites in the extracellular domain of megalin. Of these 13 sites, 9 sites within the linker sequences between the ligand-binding LA repeats were specifically lost in *Galnt11*-deficient kidneys. Analysis of other tissues with high expression of LRPs (brain and liver) revealed Galnt11-specific glycosylation in the linker regions of VLDLR, LRP8, SorLA, LRP1, LRP1B, and the LDL receptor (*SI Appendix*, Fig. S6 and Table S1), similar to what has been seen previously in cell culture (11, 12).

To further examine the role of *Galnt11* in megalin function, we created a recombinant megalin construct (Megalin-GFP) containing regions of differential glycosylation (*SI Appendix*, Fig. S7) and expressed it in HEK293 isogenic cells with (*WT*) or without (*ΔT11*) expression of *GALNT11* (12) to assess effects on ligand binding. Transfected cells in which endocytosis was blocked were incubated with labeled albumin (Alexa 647-BSA) and washed, and then bound albumin was quantitated. Albumin did not bind to either *WT* or *ΔT11* cells transfected with vector alone (GFP; Fig. 4 *C* and *D*). Interestingly, significant albumin binding was seen in *WT* cells expressing Megalin-GFP, whereas albumin binding was reduced in *ΔT11* cells expressing Megalin-GFP. These results further support a role for Galnt11 in enhancing the ability of megalin to bind ligands. Taken together, our study defines a role for Galnt11 in proper kidney function and identifies megalin as a functional substrate for Galnt11 in the proximal tubule cells of the kidney.

Discussion

Here, we provide mechanistic details regarding how Galnt11 is involved in proper kidney function and insight into the association of human GALNT11 with CKD, a major health concern worldwide. GWAS have identified numerous genes associated with idiopathic kidney disease that, if validated, could help inform the development of new treatments and methods of detection (3–5). Here, we provide evidence that the mouse ortholog of the GWAS candidate gene *GALNT11* is required for proper receptor-mediated protein reabsorption within the proximal tubules of the kidney. Loss of *Galnt11* results in low-molecular-weight proteinuria, a phenotype characteristic of defects in the endocytic receptor megalin (25, 27, 30). Indeed, we identify the endocytic receptor megalin/LRP2 (a member of the LDLR family) as a direct target of Galnt11 glycosylation in vivo. Moreover, we demonstrate a functional role for megalin glycosylation and identify *Galnt11* as a key component in proper kidney function.

Megalin is 1 of 2 multiligand receptors expressed in the proximal tubules of the kidney and is responsible for the recovery of filtered proteins such as DBP, α_1-M, and RBP (25–27, 30). In *Galnt11*$^{-/-}$ mice, reduced binding of these ligands is seen along the apical surface of the proximal tubules in vivo. Interestingly, loss of *Galnt11* resulted in decreased glycosylation of megalin at specific sites previously shown to influence ligand binding of the related LDLR and VLDLR, suggesting that glycosylation within these regions serves to modulate receptor function (11, 12, 31). This model is further supported by our data demonstrating reduced albumin binding to recombinant megalin expressed in cells deficient for *GALNT11*. Taken together, these results highlight a functional role for the O-glycosylation of megalin in proper ligand binding.

Loss of *Galnt11* also resulted in an age-related decline in megalin abundance along the surface of the proximal tubules, which could contribute to kidney functional decline over time. Megalin is known to undergo regulated proteolysis, including metalloprotease-mediated ectodomain shedding and γ-secretase-mediated intramembrane proteolysis (32–34). Whether the age-related decline in megalin abundance seen in *Galnt11*-deficient animals is related to changes in ligand-binding (and subsequent endocytosis and recycling of megalin) or a result of stability/protection from proteolysis independent of ligand binding is currently unknown. Modulation of protein stability by O-glycosylation is predicted across many proteins based on bioinformatic analysis of the proximity of predicted sites of O-glycosylation to protease cleavage sites (35); some of these have been confirmed in cell culture (23). Indeed, there is precedent for O-glycans conferring

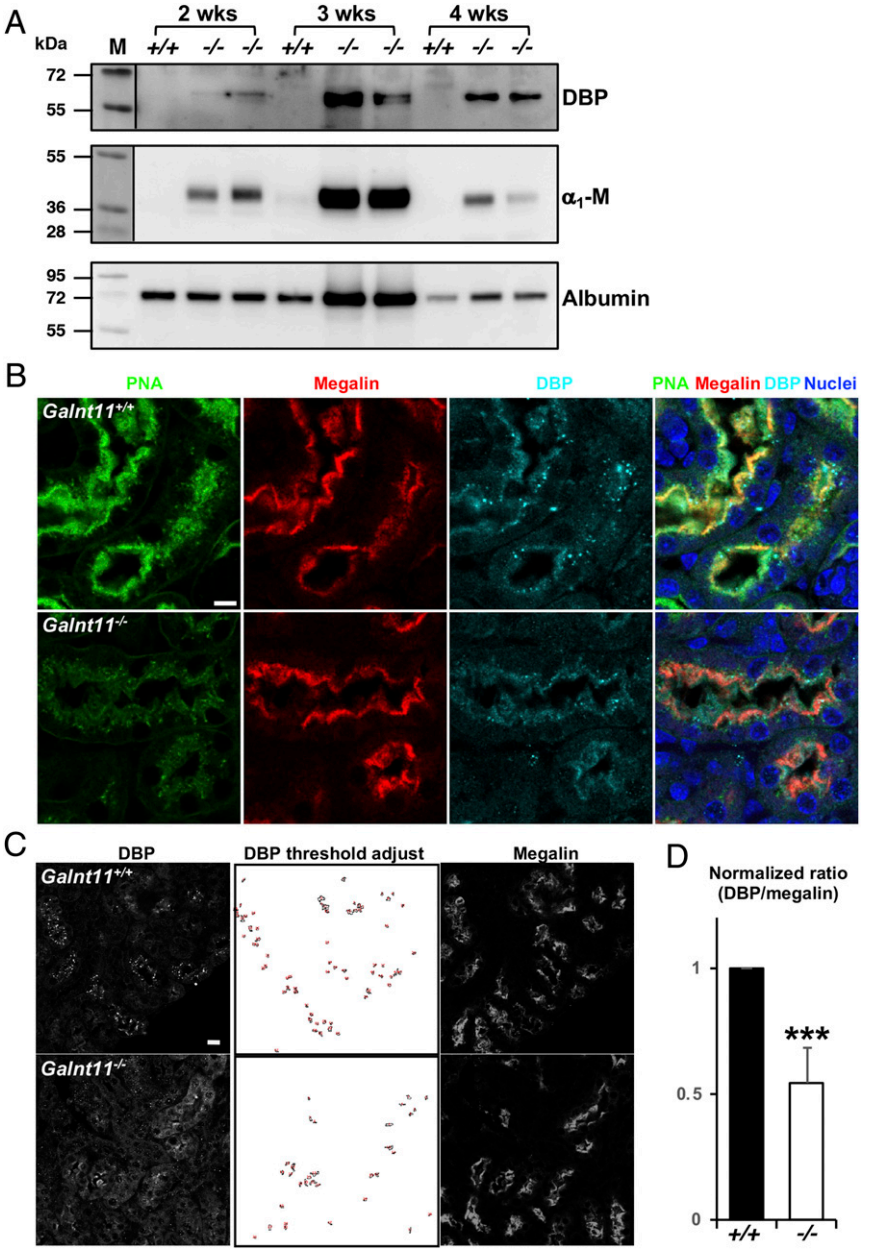

Fig. 3. Decreased ligand binding by megalin in young *Galnt11*$^{-/-}$ animals. (*A*) Young *Galnt11*$^{-/-}$ mice that express megalin along the proximal tubules still have low-molecular-weight proteinuria relative to *WT* (*Galnt11*$^{+/+}$) littermate controls. Western blots of urine from *WT* and *Galnt11*$^{-/-}$ animals were probed with antibodies to DBP, α_1-M, and albumin. All lanes in each blot are contiguous but the vertical black lines in the top two panels denote different exposures of marker and sample lanes. (*B*) Immunofluorescence reveals a specific decrease in DBP (cyan) bound to the surface of the proximal tubules in *Galnt11*$^{-/-}$ animals relative to *WT* littermate controls at 2 wk of age. Megalin (red) is present along the apical surface of the proximal tubules in *Galnt11*$^{-/-}$ animals at this age, but O-glycans (as detected by the lectin PNA, green) are not. Nuclear staining is shown in dark blue. (*C*) Quantitation of DBP binding relative to megalin levels along the apical surface is shown for each genotype. (*D*) Normalized ratios of DBP/megalin in *WT* and *Galnt11*$^{-/-}$ proximal tubules are shown. ***$P < 0.001$. (Scale bars, 10 μm.)

stability within the LRP family, as altered O-glycosylation of LDLR in cell lines deficient for O-glycosylation resulted in increased proteolytic cleavage (36, 37). O-glycosylation serves a similar protective role in vivo for the hormone FGF23 in humans (38) and for the cargo receptor Tango1 in *Drosophila* (20).

Galnt11 is unique in that it is the most abundantly expressed family member in the kidney and is specifically expressed within the proximal tubules. Interestingly, the ortholog of *Galnt11* (*pgant35A*) was first identified as an essential gene in *Drosophila* and displays similar enzymatic activity and substrate preferences to its mammalian counterpart (8, 9, 12, 13). Similar to its mammalian counterpart, *pgant35A* is also expressed in tubule cells and, when disrupted, results in the loss of apical O-glycans and apical proteins (10), highlighting the phenotypic similarities between mammals and flies upon loss of this conserved glycosyltransferase. Likewise, experiments ablating the ortholog of *Galnt11* in zebrafish also point to a role in kidney function, as kidneys were more susceptible to nephrotic insults (4).

Identification of an O-glycosyltransferase as a regulator of kidney function through its modification of the endocytic receptor megalin may provide clues as to the cause of certain idiopathic kidney diseases. Taken together, our study identifies a gene involved in kidney

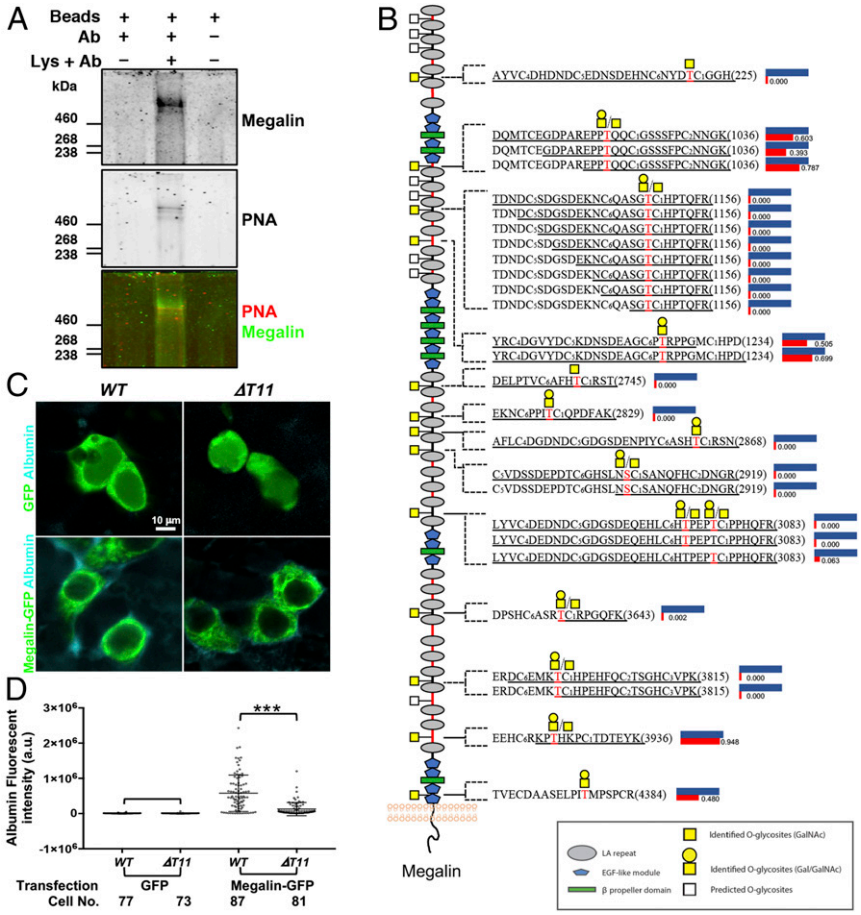

Fig. 4. Galnt11 glycosylate-specific sites within the extracellular region of megalin. (*A*) Immunoprecipitation shows that megalin from *WT* kidneys is O-glycosylated (as detected by the lectin PNA). Lys, lysate; Ab, anti-megalin antibody. (*B*) Mass spectrometry analysis of megalin/LRP2 from *WT* and *Galnt11*$^{-/-}$ kidneys shows specific sites of O-glycosylation of megalin that are altered in the absence of *Galnt11*. The right side depicts specific glycopeptides identified by mass spectrometry and the differential quantification in *WT* (denoted by the blue bar and set to 1) compared with *Galnt11*$^{-/-}$ mice (denoted by the red bar, followed by numerical occupancy relative to *WT*). Amino acids modified by GalNAc (yellow square) or the disaccharide Galβ1,3GalNAc (yellow circle and yellow square) are colored red. LA linkers with the sequence motif XX-C6(X)nTC1-XX ($n = 3$ to 5) are indicated as black lines and other linkers with red lines. Summary of identified (filled yellow squares) or predicted (by the NetOGlyc 4.0 server; open squares) O-glycosites is shown to the left. (*C*) *WT* or *GALNT11-deficient* (*ΔT11*) HEK cells were transfected with GFP vector alone (green) or Megalin-GFP (green), and albumin (Alexa 647-albumin; blue) binding was assayed. (*D*) Quantitation of albumin binding per cell in *WT* or *ΔT11* cells transfected with GFP vector or Megalin-GFP. Note increased albumin binding by Megalin-GFP in *WT* relative to *ΔT11* cells. ***$P < 0.001$.

function and provides mechanistic insight that could inform future clinical treatments and diagnoses.

Materials and Methods

Mouse Strains and Genotyping. The Animal Care and Use Committee of the National Institutes of Health (ASP protocol #17-833) approved all procedures (described in the *SI Appendix*).

Quantitative Real-Time PCR. Primers used are as described previously (18); additional information is in the *SI Appendix*.

Paraffin In Situ Hybridization. *Galnt11* RNA probes were prepared as described previously (18) and found in the *SI Appendix*.

Immunofluorescence. Immunofluorescence was performed as described previously (18) and described in the *SI Appendix*.

Microalbuminuria Enzyme-Linked Immunosorbent Assay. Details are described in the *SI Appendix*.

Immunoprecipitation and Western Blotting. Details are described in the *SI Appendix*.

Plasma Cholesterol Detection. Details are described in the *SI Appendix*.

Tissue Homogenization, Trypsinization, and Neuraminidase Treatment. Details are described in the *SI Appendix*.

Dimethyl-Labeling, Lectin Weak-Affinity Chromatography (LWAC), Mass Spectroscopy and Data Analysis. Details are as performed previously (12) and described in the *SI Appendix*. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (39) with the dataset identifier PXD015429 (29).

Megalin Construction and Binding Assays. Details are described in the *SI Appendix*.

Statistical Analysis. Details are described in the *SI Appendix*.

Data Availability Statement. All data discussed in the paper is available in the *SI Appendix*.

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1. J. Coresh *et al*., Prevalence of chronic kidney disease in the United States. *J. Am. Med. Assoc.* **298**, 2038–2047 (2007).
2. A. Meguid El Nahas, A. K. Bello, Chronic kidney disease: The global challenge. *Lancet* **365**, 331–340 (2005).
3. D. I. Chasman *et al*.; CARDIoGRAM Consortium; ICBP Consortium; CARe Consortium; WTCCC2, Integration of genome-wide association studies with biological knowledge identifies six novel genes related to kidney function. *Hum. Mol. Genet.* **21**, 5329–5343 (2012).
4. M. Gorski *et al*., Genome-wide association study of kidney function decline in individuals of European descent. *Kidney Int.* **87**, 1017–1029 (2015).
5. A. Parsa *et al*., Common variants in Mendelian kidney disease genes and their association with renal function. *J. Am. Soc. Nephrol.* **24**, 2105–2117 (2013).
6. E. P. Bennett *et al*., Control of mucin-type O-glycosylation: A classification of the polypeptide GalNAc-transferase gene family. *Glycobiology* **22**, 736–756 (2012).
7. D. T. Tran, K. G. Ten Hagen, Mucin-type O-glycosylation during development. *J. Biol. Chem.* **288**, 6921–6929 (2013).
8. T. Schwientek *et al*., Functional conservation of subfamilies of putative UDP-N-acetylgalactosamine:polypeptide N-acetylgalactosaminyltransferases in Drosophila, Caenorhabditis elegans, and mammals. One subfamily composed of l(2)35Aa is essential in Drosophila. *J. Biol. Chem.* **277**, 22623–22638 (2002).
9. K. G. Ten Hagen, D. T. Tran, A UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase is essential for viability in Drosophila melanogaster. *J. Biol. Chem.* **277**, 22616–22622 (2002).
10. E. Tian, K. G. Ten Hagen, A UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase is required for epithelial tube formation. *J. Biol. Chem.* **282**, 606–614 (2007).
11. N. B. Pedersen *et al*., Low density lipoprotein receptor class A repeats are O-glycosylated in linker regions. *J. Biol. Chem.* **289**, 17312–17324 (2014).
12. S. Wang *et al*., Site-specific *O*-glycosylation of members of the low-density lipoprotein receptor superfamily enhances ligand interactions. *J. Biol. Chem.* **293**, 7408–7422 (2018).
13. K. G. Ten Hagen, D. T. Tran, T. A. Gerken, D. S. Stein, Z. Zhang, Functional characterization and expression analysis of members of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase family from Drosophila melanogaster. *J. Biol. Chem.* **278**, 35039–35048 (2003).
14. K. G. ten Hagen, L. Zhang, E. Tian, Y. Zhang, Glycobiology on the fly: Developmental and mechanistic insights from Drosophila. *Glycobiology* **19**, 102–111 (2009).
15. E. Tian, K. G. Ten Hagen, Expression of the UDP-GalNAc: Polypeptide N-acetylgalactosaminyltransferase family is spatially and temporally regulated during Drosophila development. *Glycobiology* **16**, 83–95 (2006).
16. S. Ji *et al*., A molecular switch orchestrates enzyme specificity and secretory granule morphology. *Nat. Commun.* **9**, 3508 (2018).
17. S. Ichikawa *et al*., Ablation of the Galnt3 gene leads to low-circulating intact fibroblast growth factor 23 (Fgf23) concentrations and hyperphosphatemia despite increased Fgf23 expression. *Endocrinology* **150**, 2543–2550 (2009).
18. E. Tian, M. P. Hoffman, K. G. Ten Hagen, O-glycosylation modulates integrin and FGF signalling by influencing the secretion of basement membrane components. *Nat. Commun.* **3**, 869 (2012).
19. D. T. Tran *et al*., Multiple members of the UDP-GalNAc: Polypeptide N-acetylgalactosaminyltransferase family are essential for viability in Drosophila. *J. Biol. Chem.* **287**, 5243–5252 (2012).
20. L. Zhang *et al*., O-glycosylation regulates polarized secretion by modulating Tango1 stability. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 7296–7301 (2014).
21. L. Zhang, D. T. Tran, K. G. Ten Hagen, An O-glycosyltransferase promotes cell adhesion during development by influencing secretion of an extracellular matrix integrin ligand. *J. Biol. Chem.* **285**, 19491–19501 (2010).
22. L. Zhang, B. Turner, K. Ribbeck, K. G. Ten Hagen, Loss of the mucosal barrier alters the progenitor cell niche via Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling. *J. Biol. Chem.* **292**, 21231–21242 (2017).
23. C. K. Goth *et al*., A systematic study of modulation of ADAM-mediated ectodomain shedding by site-specific O-glycosylation. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 14623–14628 (2015).
24. K. T. Schjoldager *et al*., A systematic study of site-specific GalNAc-type O-glycosylation modulating proprotein convertase processing. *J. Biol. Chem.* **286**, 40122–40132 (2011).
25. J. R. Leheste *et al*., Megalin knockout mice as an animal model of low molecular weight proteinuria. *Am. J. Pathol.* **155**, 1361–1370 (1999).
26. M. P. Marzolo, P. Farfán, New insights into the roles of megalin/LRP2 and the regulation of its functional expression. *Biol. Res.* **44**, 89–105 (2011).
27. K. Weyer *et al*., Mouse model of proximal tubule endocytic dysfunction. *Nephrol. Dial. Transplant.* **26**, 3446–3451 (2011).
28. K. T. Schjoldager *et al*., Deconstruction of O-glycosylation–GalNAc-T isoforms direct distinct subsets of the O-glycoproteome. *EMBO Rep.* **16**, 1713–1722 (2015).
29. K. Schjoldager, Galnt11 differential tissue glycoproteomics. ProteomeXchange Repository. http://www.ebi.ac.uk/pride/archive/projects/PXD015429. Deposited 12 September 2019.
30. R. Nielsen, E. I. Christensen, H. Birn, Megalin and cubilin in proximal tubule protein reabsorption: From experimental models to human disease. *Kidney Int.* **89**, 58–67 (2016).
31. Y. Narimatsu *et al*., Exploring regulation of protein O-glycosylation in isogenic human HEK293 cells by differential O-glycoproteomics. *Mol. Cell Proteomics* **18**, 1396–1409 (2019).
32. D. Biemesderfer, Regulated intramembrane proteolysis of megalin: Linking urinary protein and gene regulation in proximal tubule? *Kidney Int.* **69**, 1717–1721 (2006).
33. Z. Zou *et al*., Linking receptor-mediated endocytosis and cell signaling: Evidence for regulated intramembrane proteolysis of megalin in proximal tubule. *J. Biol. Chem.* **279**, 34302–34310 (2004).
34. M. Shah, O. Y. Baterina, Jr, V. Taupin, M. G. Farquhar, ARH directs megalin to the endocytic recycling compartment to regulate its proteolysis and gene expression. *J. Cell Biol.* **202**, 113–127 (2013).
35. K. T. Schjoldager, H. Clausen, Site-specific protein O-glycosylation modulates proprotein processing–Deciphering specific functions of the large polypeptide GalNAc-transferase gene family. *Biochim. Biophys. Acta* **1820**, 2079–2094 (2012).
36. K. Kozarsky, D. Kingsley, M. Krieger, Use of a mutant cell line to study the kinetics and function of O-linked glycosylation of low density lipoprotein receptors. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4335–4339 (1988).
37. D. M. Kingsley, K. F. Kozarsky, L. Hobbie, M. Krieger, Reversible defects in O-linked glycosylation and LDL receptor expression in a UDP-Gal/UDP-GalNAc 4-epimerase deficient mutant. *Cell* **44**, 749–759 (1986).
38. O. Topaz *et al*., Mutations in GALNT3, encoding a protein involved in O-linked glycosylation, cause familial tumoral calcinosis. *Nat. Genet.* **36**, 579–581 (2004).
39. Y. Perez-Riverol *et al*., The PRIDE database and related tools and resources in 2019: Improving support for quantification data. *Nucleic Acids Res.* **47**, D442–D450 (2019).