Abolishment of proximal tubule albumin endocytosis does not affect plasma albumin during nephrotic syndrome in mice

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The megalin/cubilin receptor complex is required for proximal tubular endocytosis and degradation of filtered albumin. An additional high-capacity retrieval pathway of intact albumin for the recovery of large amounts of filtered albumin has been proposed, possibly involving cooperation between megalin/cubilin and the neonatal Fc receptor. To clarify the potential role of such a pathway, we examined the effects of megalin/cubilin gene inactivation on tubular albumin uptake and plasma albumin levels in nephrotic, podocin knockout mice. Immunofluorescence microscopy of megalin/cubilin/podocin knockout mouse kidneys demonstrated abolishment of proximal tubule albumin uptake, in contrast to the excessive albumin accumulation observed in podocin knockout mice compared to controls. Correspondingly, urinary albumin excretion was increased 1.4 fold in megalin/cubilin/podocin compared to podocin knockout mice (albumin/creatinine: 226 vs. 157 mg/mg). However, no difference in plasma albumin levels was observed between megalin/cubilin/podocin and podocin knockout mice, as both were reduced to approximately 40% of controls. There were no differences in liver albumin synthesis by mRNA levels and protein abundance. Thus, megalin/cubilin knockout efficiently blocks proximal tubular albumin uptake in nephrotic mice but plasma albumin levels did not differ as a result of megalin/cubilin-deficiency, suggesting no significance of the megalin/cubilin-pathway for albumin homeostasis by retrieval of intact albumin.

Kidney International (2017) ■, ■–■; http://dx.doi.org/10.1016/j.kint.2017.07.024

KEYWORDS: albuminuria; endocytosis; nephrotic syndrome; proteinuria; proximal tubule

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Received 11 June 2017; revised 11 July 2017; accepted 27 July 2017

The renal handling of albumin, the quantitatively most important plasma protein, has been rigorously investigated. Increased urinary excretion of albumin, i.e., albuminuria, is an early manifestation and important prognostic marker in various forms of kidney disease.[1] Nevertheless, several aspects of renal albumin handling remain controversial, in particular with regard to the amount of albumin filtered by the normal glomeruli and its route of reabsorption, degradation, and potential transcytosis in the proximal tubule cells. Understanding the contribution of these pathways is important for the elucidation of the pathophysiological mechanisms of albuminuria and its potentially harmful role in the progression of kidney diseases.[2]

The prevailing view is that normal glomerular filtration of albumin is limited by the properties of the glomerular filtration barrier to 0.5 to 2 g/d in humans and that the filtered albumin is efficiently reabsorbed and degraded by the proximal tubule.[3] This perception has been challenged by the proposal that large amounts of albumin are normally filtered in the kidney (~225 g/d) and that a high-capacity retrieval pathway in the proximal tubule returns albumin to the circulation to maintain plasma albumin concentrations.[4,5]

The proximal tubule cell is responsible for renal reabsorption of all filtered proteins, including albumin, primarily mediated by the megalin/cubilin receptor complex.[6–12] Following megalin/cubilin-mediated endocytosis, albumin is targeted for lysosomal degradation, whereas the receptors recycle to the apical plasma membrane. It has been suggested that albumin can also be rescued by the neonatal Fc receptor (FcRn), leading to transcytosis and the return of intact albumin to the circulation.[13,14] As FcRn knockout (KO) mice develop hypoalbuminemia,[15] FcRn-mediated transcytosis of albumin has been claimed to have a crucial role in maintaining normal plasma albumin levels.[16] FcRn binds albumin with higher affinity at low pH (pH 6.1),[17] allowing it to bind albumin in the acidic environment of the endosome and releasing it at a higher pH in the extracellular environment. Because the pH of the proximal tubule lumen does not facilitate albumin binding to FcRn, it was further suggested that the receptor pathways cooperate in the transcellular

transport of albumin.[13] This implies that the megalin/cubilin receptor pathway is responsible for the initial binding, endocytosis, and transport of albumin to the early endosomes. The acidic pH of this compartment causes dissociation of albumin from the megalin/cubilin receptors and binding to the FcRn, mediating transport across the proximal tubule cells and release at the basolateral surface. This hypothesis implies that megalin/cubilin-mediated albumin uptake contributes to the retrieval of intact albumin and thereby preservation of plasma albumin levels. To address this, we investigated proximal tubular albumin uptake and plasma albumin levels when megalin/cubilin-mediated uptake of albumin was abolished in normal and nephrotic mice using novel, inducible KO mouse models.

RESULTS

Generation of inducible nephrotic megalin/cubilin receptor KO mice

Tamoxifen treatment of inducible megalin/cubilin, megalin/cubilin/podocin, and podocin KO mice resulted in efficient (~85%–92%) inactivation of all the respective genes in the kidney (Figure 1a–c). This was confirmed by immunohistochemistry, showing the almost complete absence of megalin and cubilin staining in proximal tubule cells and podocin

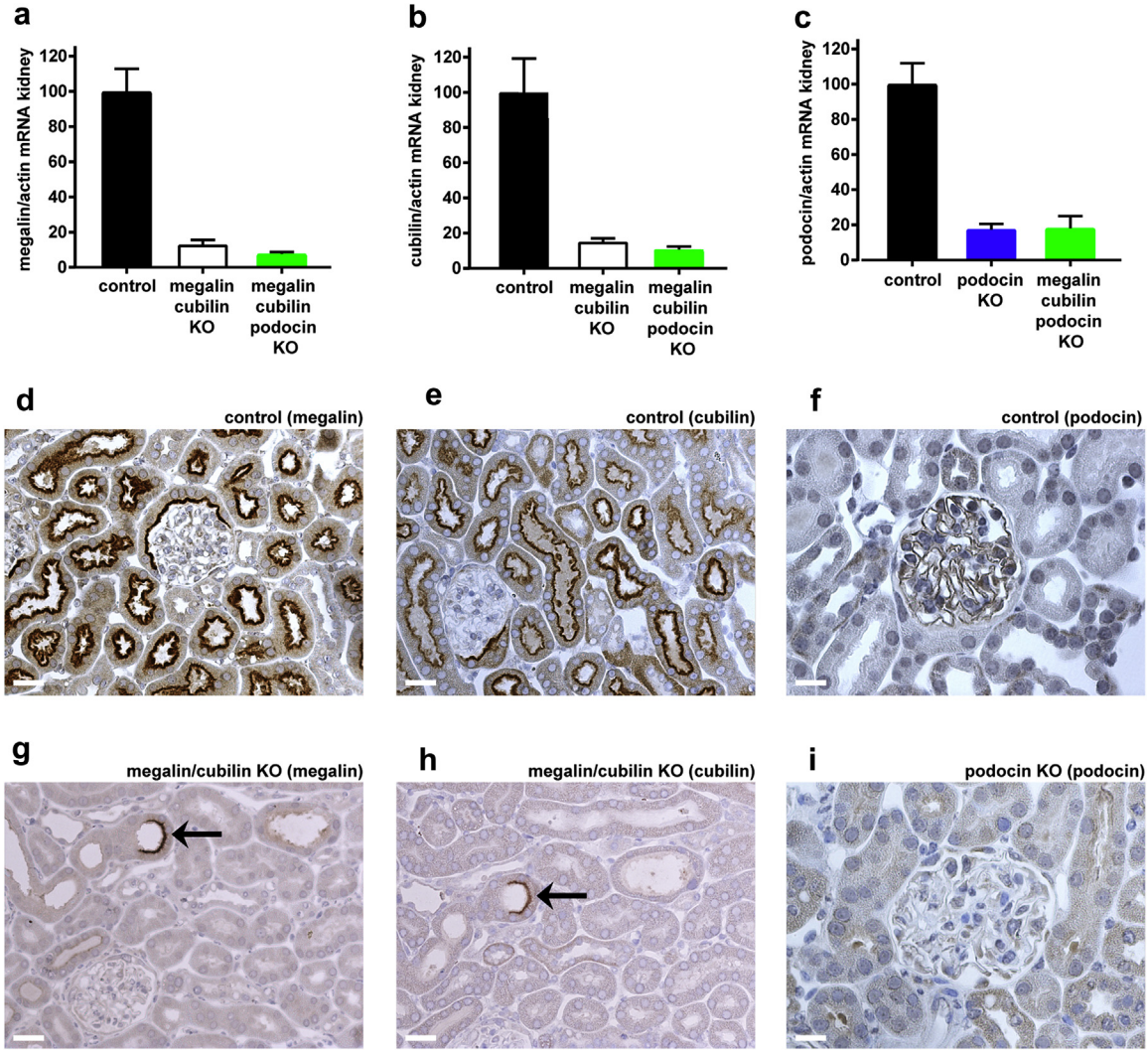

Figure 1 | Megalin/cubilin/podocin gene inactivation in the kidney by quantitative real-time PCR (qRT-PCR) and immunohistochemistry. (**a–c**) The relationship of megalin, cubilin, and podocin to the actin mRNA ratio from inducible megalin/cubilin knockout (KO) mice, megalin/cubilin/podocin KO mice, and podocin KO mice compared with control mice, determined by qRT-PCR using whole kidney samples. The graphs show the mean values from all mice in each group ($N = 8$), and error bars indicate ± SEM. (**d–i**) Immunohistochemical labeling for megalin, cubilin, and podocin in kidney sections from control, inducible megalin/cubilin KO, or inducible podocin KO mice, as indicated above the images. (**g**,**h**) Arrows point out residual expression of megalin and cubilin in a few mosaic tubules in serial sections from kidneys of inducible megalin/cubilin KO mice. Note that the megalin/cubilin receptors appear to be knocked out together in the proximal tubule cells. (**d**,**e**,**g**,**h**) Bars = 50 μm. (**f**,**i**) Bars = 25 μm. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.