DACH1 as a multifaceted and potentially druggable susceptibility factor for kidney disease

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Kidney diseases affect more than 15% of adults in the US, yet drug development in the kidney field, when compared with that for other common diseases, has been lagging behind. Modifiers that increase the susceptibility to injury and contribute to the pathogenesis and progression of kidney disease include genetic and environmental factors and epigenetic mechanisms. In this issue of the *JCI*, Cao et al. and Doke et al. independently report the identification of a susceptibility factor called Dachshund homolog 1 (DACH1). Both groups identify an association of reduced DACH1 expression with kidney disease, using different screening approaches, studying different types of human kidney diseases, and using different experimental models, making the fact that both stumbled over the same protein very compelling. Combined, these studies highlight DACH1 as a key safeguard in the kidney, granting various cell types proper function by modulating several molecular pathways.

Getting a grip on kidney disease

Diabetes, high blood pressure, and a family history of kidney disease are major risk factors contributing to the high prevalence of kidney disease. While the genetic origins of kidney diseases have been extensively studied in the past, research in recent years has shifted toward a better understanding of the epigenetic components that make the kidney more vulnerable to consecutive insults. For example, hyperglycemia in patients with diabetes, most of whom develop kidney disease over time, induces epigenetic changes and renders the kidney susceptible to injury (1). One way of getting a better grip on understanding the mechanisms contributing to kidney disease pathogenesis is to identify proteins within kidney cells that have universal cell-protective effects, mediate injury resistance, and safeguard the kidney from further insults. One could hypothesize that the reduced expression or function of such safeguards, for example, by genetic or epigenetic modifications, contributes to kidney injury, while maintaining their proper physiological activity might serve as a pharmacological opportunity for preserving kidney function. If true, genetic variants of such safeguards should associate with kidney disease.

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Conflict of interest: SM is an inventor on pending patents (PCT/US2019/032215 and US17/057,247: Methods of treating renal disease associated with chronic kidney disease such as Alport syndrome; PCT/US2019/041730 and US17/259,883: Japan no. 501309/2021, Europe no. 19834217.2, and China no. 201980060078.3: Method for treating kidney disorders; PCT/US2013/036484: Method of using cyclodextrin; CAN 2,930,119 and 3,012,773: Assays, methods and kits for predicting renal disease and personalized treatment strategies) and issued patents (US 10,183,038: Method for preventing and treating renal disease; US 10,052,345: Assays, methods and kits for predicting renal disease and personalized treatment strategies; CAN 2,852,904: Assays, methods and kits for predicting renal disease and personalized treatment strategies). She stands to gain royalties from their future commercialization. SM holds indirect equity interest in and potential royalty from ZyVersa Therapeutics Inc. by virtue of assignment and licensure of a patent estate. SM has served as a consultant for Kintai Therapeutics Inc. CF has served as a consultant for Bayer and Calico Labs. He is an inventor on two pending patents (PCT/US2019/049211: Production and detection of bioactive soluble klotho protein; PCT/US2019/049161: Drug screening for FGF23/FGFR4 inhibitors), and he has one patent (European patent no. 2723391: Fibroblast growth factor receptor inhibition for the treatment of disease). He is a cofounder and the CSO of a startup biotech company (Alpha-Young LLC).

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DACH1 and the kidney

Dachshund homolog 1 (DACH1) transcriptionally represses specific target genes by either directly binding to defined DNA sequences or indirectly acting as a cointegrator for other transcription factors. DACH1 is widely expressed in normal adult tissues and has various functions. DACH1 acts as a transcriptional repressor of cell-cycle genes and as a tumor suppressor, and reduced expression levels of DACH1 correlate with poor prognosis in various types of cancer. DACH1 has also been shown to interact with components of the TGF-β signaling pathway and to promote epithelial-to-mesenchymal transition. During embryonic development, DACH1 plays a role in cell-fate determination, and studies in humans demonstrated that rare loss-of-function mutations in *DACH1* can cause numerous congenital anomalies, including kidney developmental defects (2, 3). Mice with a homozygous *Dach1* deletion die early, although no morphological or metabolic alterations in any organs were detected (4). In this issue of the *JCI*, Cao et al. report the development of *Dach1*-deficient mice, which were born at expected Mendelian ratios, but died two days after birth, presenting with renal hypoplasia and dramatic glomerular anomalies, including podocyte maturation failure in the absence of gross abnormalities in other major organs (5).

DACH1 and podocyte injury

Podocytes, together with the glomerular basement membrane and the fenestrated

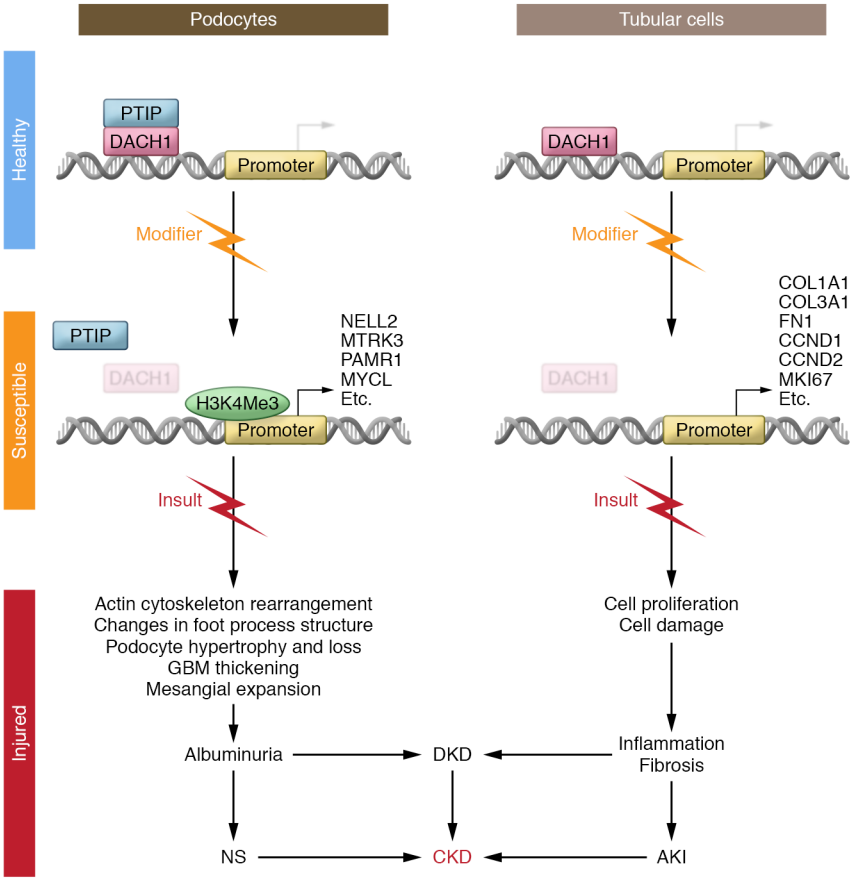

Figure 1. Decreased podocyte and tubular DACH1 expression is a susceptibility factor for various kidney diseases. In podocytes (left), DACH1 is a safeguard in the nucleus that limits histone H3 lysine 4 trimethylation (H3K4Me3), thereby preventing transcriptional activation of several genes. DACH1 interacts with pax transactivation-domain interacting protein (PTIP), an essential component of the H3K4Me3 complex, which results in reduced promoter methylation and transcriptional repression. Modifiers, which can be of genetic, epigenetic, or environmental origin, can lead to reduced DACH1 expression and to transcriptional derepression of target genes, making podocytes more susceptible to a second insult. A second insult, such as high glucose levels, may ultimately cause podocyte injury and albuminuria. In tubular cells (right), DACH1 acts as a transcriptional suppressor of cell-cycle genes and of proinflammatory cytokines. Modifiers leading to reduced tubular DACH1 expression and to transcriptional derepression of target genes make the tubular cells more susceptible to a second insult, such as folic acid–induced kidney injury or hyperglycemia, ultimately causing tubular cell proliferation or injury and eventually renal inflammation and fibrosis. For both cell types, modifiers and mechanisms that induce the reduction of DACH1 expression are currently unknown.

endothelium, build the glomerular filtration barrier. Podocyte injury is a hallmark of many kidney diseases, including nephrotic syndrome and diabetic kidney disease (DKD), leading to the loss of albumin in the urine (albuminuria).

A previous study demonstrated a crucial role for DACH1 in zebrafish nephrocyte development and as an inducer of podocyte-specific proteins during differentiation (6). Furthermore, DACH1 seems to contribute to cell-cycle arrest in podocytes that are postmitotic (7). Several genome-wide association studies (GWAS) found that a SNP in the *DACH1* locus is associated with reduced kidney function and chronic kidney disease (CKD) (8–11), and *DACH1* has been identified as a promising candidate gene for therapeutic intervention in CKD (12).

Cao and colleagues identified DACH1 as a major regulator of podocyte structure and function (5). Analyzing available glomerular transcriptomic data sets, they found reduced *DACH1* mRNA levels in different human glomerular diseases, including several forms of nephrotic syndrome and particularly in patients with DKD, an observation that is consistent with a previous report demonstrating reduced podocyte DACH1 expression levels in similar patient populations (6, 7). More importantly, Cao et al. demonstrate that reduced glomerular *DACH1* mRNA correlates with poor clinical outcomes, such as albuminuria and reduced kidney function. To determine a potential causative role of podocyte DACH1 in kidney injury, the authors developed two mouse models for the podocyte-specific deletion and inducible overexpression of DACH1. Both models per se failed to develop a phenotype and maintained normal glomerular architecture. However, the mice exhibited different responses to hyperglycemia-induced injury. Mice with podocyte *Dach1* deficiency, which per se do not show kidney injury, developed podocyte injury and albuminuria following streptozotocin (STZ) injections. In contrast, inducible overexpression of *Dach1* in OVE26 mice, a transgenic model of severe early onset hyperglycemia, protected from glomerular injury and albuminuria. These studies suggest an important role for podocyte DACH1 as a susceptibility factor in hyperglycemia-induced kidney injury (Figure 1). To investigate a podocyte-specific role of DACH1 in nephrotic syndrome, the authors injected *Dach1*-deficient mice with adriamycin, a recognized agent that induces podocyte injury, and found that *Dach1* deficiency rendered mice susceptible to adriamycin-induced nephropathy (5).

DACH1 and renal tubular injury

In this issue of the *JCI,* Doke et al. also identified *DACH1* as a candidate gene for kidney disease using a powerful approach that included transcriptome-wide association studies (TWAS), single-cell epigenome analysis, and GWAS in combination with expression analysis, gene editing, and functional validation (13). Using CRISPR-Cas9 gene-editing technology in distal tubular cells, the researchers validated and defined the causal role of an estimated glomerular filtration rate (eGFR) GWAS SNP in regulating DACH1 expression in tubular cells. Analysis of DACH1 expression by immunohistochemistry indicated that DACH1 levels were decreased in the distal tubular segments in kidneys of patients with CKD. Gene expression analysis of microdissected human kidney tubule samples revealed

a positive correlation of DACH1 expression with kidney function and a negative correlation with kidney fibrosis, inflammation, and cell proliferation (13).

To determine a potential causative role of tubular DACH1 in kidney injury, Doke et al. generated two mouse models, one with tubule-specific *Dach1* deletion and another with inducible overexpression (13). Similarly to what was found in the study by Cao et al., which analyzed podocyte-specific *Dach1* deletion and overexpression (5), mice with tubular *Dach1* deletion or overexpression did not develop a phenotype per se (13). However, compared with control animals, the mice responded differently to injection of folic acid, a model of acute kidney injury (AKI), i.e., mice with tubule-specific *Dach1* deficiency developed severe renal fibrosis and tubular damage, while mice with tubule-specific *Dach1* overexpression were protected from folic acid–induced nephropathy. Similarly, STZ injections combined with uninephrectomy in mice with tubule-specific *Dach1* deficiency were associated with increased renal fibrosis and proteinuria. Taken together, the studies by Cao et al. and Doke et al. demonstrate that reduced tubular DACH1 expression levels predispose to kidney injury, while increased tubular DACH1 expression levels are renoprotective, suggesting a role of tubular DACH1 in kidney disease (5, 13) (Figure 1).

DACH1, a safeguard and drug target?

It is interesting that two independent studies identify DACH1 as a susceptibility factor for kidney diseases, including DKD and nephrotic syndrome, although each group focused on a different cell type, i.e., podocytes or tubular cells (5, 13). The finding that diminished DACH1 expression in either cell type is insufficient to cause injury, but renders the cells susceptible to injury, supports the hypothesis of a multihit injury process in the pathogenesis of kidney diseases. Of note, a previous in vitro mutagenic screen with the goal of identifying susceptibility genes for HIV-associated nephropathy (HIVAN), which is characterized by podocyte proliferation, also detected reduced DACH1 expression (14). This finding is consistent with the well-established role of DACH1 as a tumor suppressor in other cell types. Podocytes are postmitotic and, within the spectrum of kidney diseases, cell-cycle reentry of podocytes is only specific to HIVAN and not to other kidney diseases, such as nephrotic syndrome or DKD. Thus, it remains unclear why in the latter diseases reduced DACH1 expression does not result in podocyte proliferation. Furthermore, it would be interesting to determine whether loss of DACH1 in podocytes results in dedifferentiation, as suggested by others (6), thereby contributing to podocyte dysfunction and loss, as found in diabetes and nephrotic syndrome. The observed severity of podocyte injury induced by STZ in podocyte-specific *Dach1*-deficient mice is surprising and reminiscent of a specific form of nephrotic syndrome rather than DKD.

The observation that tubular reduction of DACH1 and of its activity as a transcriptional suppressor is associated with a proinflammatory tubular cell phenotype and cytokine release, leading to macrophage infiltration, fibrosis, and CKD development, is interesting. However, the mechanisms by which changes in tubular DACH1 expression lead to kidney fibrosis and tubular damage remain to be established. Furthermore, since glomerular injury likely precedes tubular injury in DKD and nephrotic syndrome, it would be interesting to determine whether a potential crosstalk between podocytes and tubular cells exists. If so, one might expect that podocyte-specific *Dach1* deletion and induced glomerular injury would result in tubular damage, which might or might not involve a reduction of tubular DACH1 expression. Furthermore, the potential effects of increased tubular cell proliferation on kidney structure and function remain unclear. A previous in vitro study reported that high glucose induces proliferation, apoptosis, and an inflammatory response in tubular cells with reduced DACH1 expression (15), which is consistent with the findings by Doke et al. (13). The authors also found that miR-218, which is elevated in DKD, negatively regulates DACH1 (15). Since miR-218 inhibits glucose uptake in cancer (16), it would be interesting to investigate the direct or indirect effects of DACH1 on glucose uptake in renal cells, for example, by regulating SGLT2.

Doke et al. identified *DACH1* as a potential candidate gene in a genetic screen of patients with CKD while experimental studies were performed in folic acid–injected mice, a model of AKI and kidney fibrosis (13). Thus, further studies in mouse models of CKD are needed to investigate the role of tubular DACH1 during chronic disease progression. It is also important to note that DACH1 expression under the control of the Pax8 promoter will increase DACH1 levels in proximal and distal tubules and the entire collecting duct system, while in human CKD, DACH1 reduction seems restricted to the distal tubules. Therefore, the phenotypes detected in this particular mouse model might not accurately reflect the human pathology.

While further experiments using animal models that better reflect human kidney disease are needed, both studies clearly demonstrate that reduced renal DACH1 expression renders kidney cells, such as podocytes and tubular cells, susceptible to injury induced by consecutive insults, highlighting the possibility that DACH1 could serve as a universal safeguard of the kidney. Because restoring DACH1 expression in podocytes or tubular cells alone is sufficient to reduce kidney injury in mice, restoring physiological DACH1 protein levels might represent a desirable therapeutic goal. However, before pharmacological targeting of DACH1 can be considered as a valid therapeutic option, we have to gain a better understanding of the mechanisms leading to a reduction of renal DACH1 expression in disease. Furthermore, delivery of DACH1 DNA, mRNA, or protein to the kidney and to specific cell types may prove challenging. The observation that DACH1 regulates many different genes and pathways and is widely expressed in different cell types may render DACH1 targeting for therapeutic purposes difficult, as unwanted potential off-target effects are possible. In general, transcription factors and epigenetic regulators, although involved in many diseases, are barely tackled by existing therapies. On the other hand, many potential therapies for CKD have failed in clinical trials because they focus on blocking or augmenting only a single pathway, whereas CKD is a multipathway disease. In conclusion, while therapeutic targeting of DACH1 may prove difficult, it would offer the opportunity to correct multiple dysregulated signals simultane-

ously and could represent a valid therapeutic option for patients with CKD.

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