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TRPC6 in diabetic kidney disease: Good guy or bad guy?

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Abstract

An association between altered activity of TRPC6 channels with the development of various diabetic complications was previously reported. In this issue, Wang *et al.* tested the contribution of TRPC6 in type 1 diabetes setting by breeding Akita mice with TRPC6 KO mice. The authors revealed that the KO of TRPC6 decreased proteinuria and attenuated tubule injury but promoted mesangial expansion in the Akita mice. Therefore, TRPC6 has both beneficial and detrimental effects on glomeruli function.

Diabetes and its renal complications are becoming an epidemic in the U.S. population. One of the major morbidity and mortality factors confronted by patients with diabetes is an increased risk of developing diabetic nephropathy (DN), which often progresses to end-stage kidney disease. Glomeruli injury, including podocytes's damage, is considered a major contributor to diabetic kidney disease (DKD). The podocyte has become a crucial focus as a target for interventions in kidney disease due to its key role in regulating glomerular permeability. The podocyte itself expresses numerous receptors and responds to many factors known to be associated with DN. One of the main factors determining the pathological changes of glomerular morphology and permeability is linked to an elevation of podocyte intracellular calcium ($[Ca^{2+}]_i$). Transient receptor potential canonical (TRPC) channels are Ca^{2+} -permeable channels that are important in the pathogenesis of renal and cardiovascular diseases. TRPC channels, including TRPC6, are responsible for the increase of $[Ca^{2+}]_i$ in glomeruli cells in response to various stimuli, including components of the renin-angiotensin system and production of reactive oxygen species.^{1–3} An association between altered TRPC channel function/expression with the development of various diabetic complications has garnered the attention of many investigators. This is particularly important for TRPC6, which has been shown to be upregulated in glomerular disease processes, including DN.^{2–5}

In this issue of *Kidney International*, Wang *et al.*⁶ (2019) used a genetic approach to investigate the role of TRPC6 in DKD. Akita mice, which are a model of type 1 diabetes, were bred with TRPC6 global knockout (KO) mice. Generated Akita mice lacking TRPC6 (TRPC6 KO Akita) were used to test the role of TRPC6 on the functional and

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DISCLOSURE

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histopathologic features of DKD. The authors performed rigorous analyses of the TRPC6 KO Akita mice and provided compelling evidence that TRPC6 KO resulted in attenuated tubule injury and reduced proteinuria until the later stages of the disease process. Surprisingly, the KO of TRPC6 caused prominent mesangial expansion in the diabetic group, which suggests enhanced susceptibility of glomerular cell types to the adverse effects of TRPC6 KO in the setting of hyperglycemia. Importantly, the authors also attempted to delineate the molecular mechanism of this effect. First, as shown through an insulin tolerance test, the TRPC6 KO Akita mice were insulin resistant compared to the WT Akita mice. Insulin levels in the blood also tended to be lower in the TRPC6 KO Akita mice as compared to WT Akita mice. In contrast to insulin, a glucose tolerance test did not reveal a statistical difference between the groups. Therefore, the authors proposed that the knockout of TRPC6 in Akita mice reduces the expression of the insulin receptor substrate 2 and promotes insulin resistance. Subsequent studies with cultured podocytes revealed that hyperglycemia activated ASK1-p38 signaling and promoted podocyte apoptosis, both of which were attenuated by an insulin-induced activation of either the PI3-Kinase-Akt or ERK pathways. It should be noted that while it is clear that the described pathways play a role in the regulation of TRPC6, the contribution of other signaling pathways to the upregulation of TRPC6 in podocytes, including hyperglycemic conditions, are previously reported.

This is an important and timely paper that provides critical insight into the contribution of TRPC6 channels in DKD. The current hypothesis in the field is that TRPC6 knock out or inhibition should provide beneficial effects in DKD. As a result of this, multiple groups in both academia and the healthcare industry are working on testing this hypothesis and developing specific drugs targeting this pathway in DN. Several studies have reported that TRPC6 activity and its expression are altered in various *in vivo* and *in vitro* diabetic models. As an example, Sonneveld *et al.* demonstrated that podocyte TRPC6 protein expression was increased in proteinuric streptozotocin (STZ)-induced diabetic rats.⁵ *In vitro* studies further revealed that high glucose levels increased TRPC6 mRNA and protein expression, and that Ang II and inducing podocyte injury also specifically increased TRPC6 expression in cultured podocytes. In addition, our earlier studies demonstrated that TRPC6 expression and activity are upregulated in STZ-treated Dahl Salt-Sensitive (SS) rats. Furthermore, a calcium influx in podocytes was triggered by Ang II.³ To further address the contribution of this channel in the setting of DN, we recently created a TRPC6 knockout on the Dahl SS rat background (SS^{Trpc6-/-}) to test the contribution of TRPC6 in DKD.⁴ Treating Dahl SS rats with STZ generates increases in renal fibrosis, glomerular injury, and proteinuria, with changes in such renal hemodynamics as elevated renal vascular resistance and hyperfiltration, which are common features of DN. Using this model represents significant advantages for studies of DN, as most of the available diabetic models have only a limited development of glomerular and kidney injury. There was an increase in albuminuria and glomerular injury following STZ treatment, which was not statistically different between the Dahl SS and SS^{Trpc6-/-} groups. However, a higher concentration of nephrin in urine, increases in basal [Ca²⁺]_i levels and foot-process damage of podocytes during the development of DKD was attenuated in the STZ-treated SS^{Trpc6-/-} as compared with STZ-SS wild-type rats. Overall, our data indicated that the TRPC6 channel inhibition may have at least partial renoprotective effects.⁴ Importantly, the effect of the TRPC6 knock out on

podocyte and kidney injury was tested up to 11 weeks following STZ treatment and, as we have previously shown, this time frame still represents an earlier time of DKD development, as 6-week treatments with STZ cause hyperglycemia but not glomerular lesions.³ The protective role of TRPC6 in other chronic kidney diseases was also shown. As an example, Kim *et al.* recently reported that TRPC6 inactivation resulted in reduced glomerulosclerosis, ultrastructural changes, interstitial changes, and renal fibrosis in the progression of chronic puromycin aminonucleoside nephrosis in Sprague-Dawley rats, a classic model for acquired nephrotic syndromes.⁷ Notably, in the context of current study by Wang *et al.*⁶, the authors reported that TRPC6 inactivation reduced albuminuria in the chronic, but not in the acute, phase.

Several independent laboratories have reported the identification of *TRPC6* mutations associated with the autosomal dominant form of focal segmental glomerulosclerosis (FSGS). These genetic discoveries highlighted the critical importance of the podocyte and the TRPC6 channel in the maintenance of glomerular filtration. The majority of identified mutations in the TRPC6 gene result in gain-of-function mutants of this channel, and activation of the TRPC6 channel leads to excessive $[Ca^{2+}]_i$ levels and podocyte cell death. Interestingly, a recent analysis of human disease-causing TRPC6 mutations reveals loss-of-function mutation as an additional concept of hereditary FSGS⁸, which demonstrates that not only activation, but also inhibition of TRPC6 activity might lead to FSGS. It was also observed correlation between gain- or loss-of-function mutations and age of patients and it was proposed that loss of TRPC6 function might be implicated in the pathophysiology of juvenile forms of hereditary FSGS. Briefly summarizing, these findings show that it is critical to maintain the physiological concentrations of $[Ca^{2+}]_i$ required for the normal function of podocytes, and that any changes in $[Ca^{2+}]_i$ levels have detrimental effects (Figure 1).

Despite its merits, the current study also has some limitations. Unfortunately, there are no cell-type specific TRPC6 knockout mice, and in the current study, Wang *et al.* crossed Akita mice with global TRPC6 KO mice. This limitation is important not only because it makes interpretation of the data more complicated, but also because TRPC6 is a ubiquitous channel that is expressed in many cell types, including glomeruli. Thus, Graham *et al.* reported previously that TRPC6 protein expression in mesangial cells (MC) was downregulated by high glucose, and the deficiency of TRPC6 protein might contribute to the impaired Ca^{2+} signaling of the MCs seen in diabetes.⁹ It is hard to say if TRPC6 in MCs contributed to mesangial expansion in TRPC6 KO Akita mice, but this possibility should not be excluded. One other limitation of all studies using TRPC6 KO in different rat or mouse strains is that in some tissues there is compensatory upregulation of TRPC3, which could influence and potentially limit the effects of TRPC6 knockout.

The authors suggest that targeting TRPC6 to treat DN may have harmful effects. While current studies provide palpable evidence that TRPC6 KO in Akita mice exacerbates such glomerular injury as mesangial expansion, more mechanistic work is needed before translating the presented results into a complete understanding of the contribution of TRPC6 in podocytopathy in general and DKD in particular. Another important question that should be explored further is the potential role of TRPC6 in the setting of type 2 diabetes mellitus,

as DN is a common complication of both diabetes types 1 and 2. The current study focuses on a type 1 model, and research of the effects of TRPC6 in type 2 diabetes, especially *in vivo*, is limited. Briefly, current manuscript provides important insight into the field of TRPC6 channels in podocytes, but additional studies are required to completely uncover the role of this channel in DN and glomeruli diseases.

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Figure 1 |. Proposed schematic of podocyte's damage when TRPC6 activity is either increased or decreased.

Gain-of-function (GOF) mutations or activation of TRPC6 by different stimuli such as Ang II or reactive oxygen species (ROS) result in $[Ca^{2+}]_i$ overload and podocytes damage. There are also loss-of-function (LOF) mutations and certain conditions when decrease in $[Ca^{2+}]_i$ levels also has detrimental effects on podocytes.