Podocytes contribute to carnitine homeostasis via expressing a carnitine efflux transporter Slc16a9

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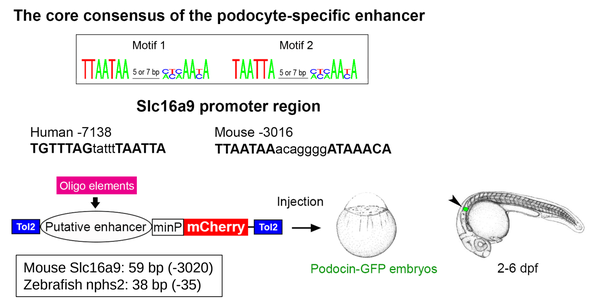
INTRODUCTION

The mechanisms underlying the increased risk of the heart disease in proteinuric patients remain poorly understood. The podocyte is the main determinant of proteinuria, a powerful marker for renal damage and cardiovascular risk. The carnitine dependence in podocytes thought to rely on glucose from glomerular filtrate as fuel and in cardiomyocytes that mainly use long chain fatty acid (LCFA) for energy is likely to differ, with a much lower requirement in podocytes. We previously predicted Slc16a9 (also known as MCT9) as a new podocyte protein due to the presence of a putative podocyte enhancer in its 5' end (He et al JASN 2014). Here we report that Slc16a9 is expressed in podocytes and contributes to the carnitine homeostasis.

METHODS

In vivo expression system

The Tol2-mediated transient transgenic zebrafish was used to evaluate putative podocyte enhancer activity.



dOligos carrying putative enhancer motifs were cloned into the Tol2-plasmid, which was then injected in 1-cell embryos. Live glomerulus was visualized under the confocal microscope.

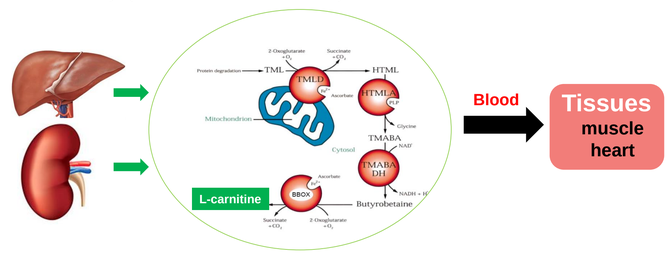
Immunofluorescence staining

The 8 µm cryosections of adult mouse kidney, liver and skeleton muscle were performed. Primary antibodies to Slc16a9 1:200 (SCT), Bbox1 1:250 (Acris) and nephrin (1:125) (Acris) were used. A negative control without primary antibodies was included.

Zeiss LSM 700 confocal microscope was applied to imaging analysis

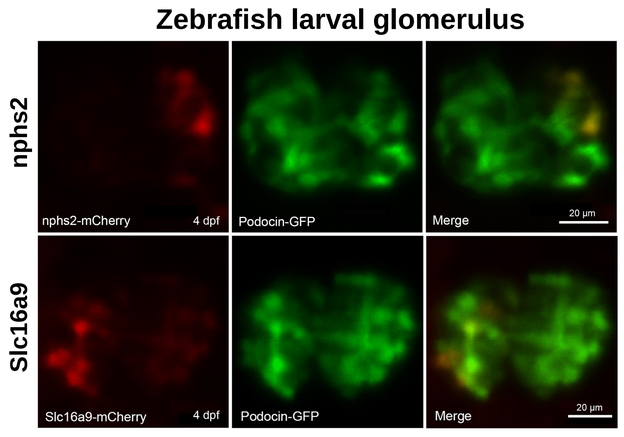
Carnitine biosynthesis

BBOX is the rate-limiting enzyme for carnitine synthesis only present in liver and kidney. (Vaz et al Biochem J 2002)



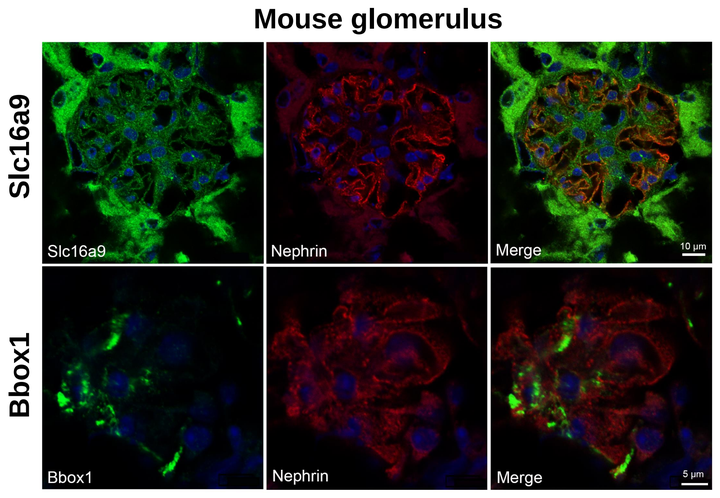
RESULTS

Zebrafish larval glomerulus



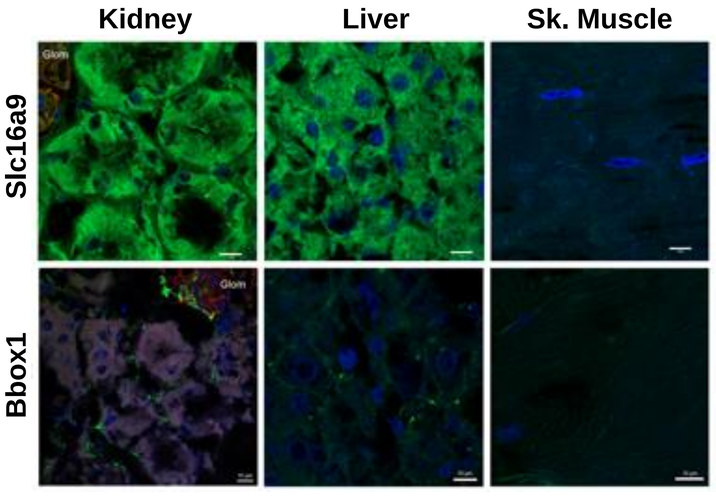
Predicted enhancer motif in mouse Slc16a9 is a cis-acting element driving mosaic mCherry expression in 4-dpf zebrafish glomerulus. Podocytes are labeled with GFP. A zebrafish nphs2 enhancer was used as a positive control.

Mouse glomerulus



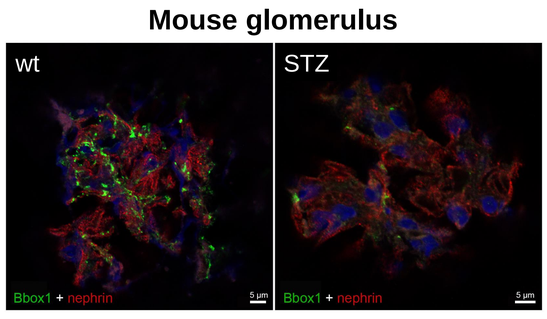
Slc16a9 (green) is expressed in podocytes co-localized with nephrin (red). The rate-limiting enzyme Bbox1 (green) for carnitine biosynthesis is also present in glomeruli.

Kidney Liver Sk. Muscle



Slc16a9 (green) is abundantly expressed in kidney tubule and liver, but absent in skeletal muscle. In parallel, Bbox1 (green) expression showed an identical pattern.

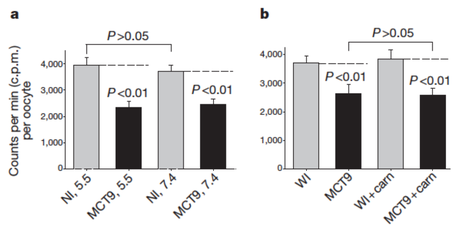
Mouse glomerulus



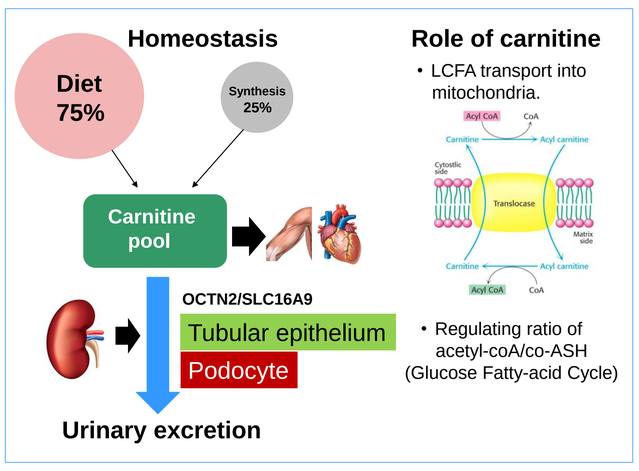
Immunofluorescence staining of kidney sections displays downregulation of Bbox1 (green) in streptozotocin (STZ)-induced diabetic mouse kidney compared to wt.

How is SLC16A9 linked to carnitine?

Blood carnitine concentration is genetically regulated. GWAS studies demonstrate that SLC16A9 variants are highly associated with plasma L-carnitine level, suggesting a biological link. (Kolz et al Plos Genet 2009; Illig et al Nat Genet 2010;Demirkan et al Plos Genet 2015)



SLC16A9 is a efflux transporter. Injected [^{3}H]-carnitine present or absent of Slc16a9 in Xenopus oocytes was counted. NI, non injected; WI, H_2O injected. (Suhre et al Nature 2011)



CONCLUSIONS

- Glomerular podocytes express a recently identified carnitine efflux transporter SLC16A9 regulated by the podocyte enhancer, suggesting an intrinsic couple of carnitine synthesis with its export.
- Expression of BBOX1 is highly correlated with its efflux transporter SLC16A9, where kidney and liver, two only organs producing carnitine, express both, but skeletal muscle is absent for both.
- High glucose seems to downregulate glomerular BBOX expression.
- Our preliminary results suggest a novel pathway linking the podocytes and the heart, where the carnitine produced and exported from podocytes contribute to cardiac energy metabolism.

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