renal blood flow and a decreased GFR.[7] Aortic flow reversal is correlated with PWV, aortic distensibility, augmentation index, aortic arch length, and aortic diameter, with aortic diameter having the strongest predictive value.[8] *Eln*+/− aortas have a decreased diameter, an increased length, and decreased distensibility as early as P7,[1] which may all contribute to altered pulsatile pressure and flow in the renal vasculature and reduced renal blood flow that lead to the observed changes in renal vascular tone, RVR, and kidney structure and function.[3]

The data presented by Owens *et al.*[3] suggest that elastin deficiency and the resulting alterations in large- and small-artery hemodynamics cause compromised kidney function independent of the effects on individual blood pressure metrics (i.e., systolic or mean pressure). The study also suggests that elastin deficiency may be a cause and not a consequence of CKD. It is important to remember, however, that the relationship is probably not unidirectional. Elastin degradation may contribute to CKD through alterations in large and small arteries, but CKD also likely contributes to elastin degradation in a positive feedback cycle. Individuals with CKD have higher serum levels of elastin-derived peptides, indicative of elastin degradation, than healthy controls.[9] There is a significant temporal relationship between changes in serum elastin–derived peptide concentration and PWV, and elastin-derived peptide levels are independently associated with all-cause mortality in CKD patients. Inflammation, oxidative stress, and mineral imbalance due to CKD have been suggested as factors leading to the observed elastin degradation, increased PWV, and resulting cardiovascular disease.

Owens *et al.*[3] provide insight into the interrelationships between CKD, hypertension, and vascular hemodynamics in the context of elastin insufficiency, as summarized in Figure 1. Important questions to be addressed in future work include experimental measurements or computational simulations of the pressure and flow waveforms at different arterial locations in the mouse to address hypotheses about altered wave reflections. The stimuli leading to altered mechanosensitivity of AT1R in the renal vasculature are unknown and represent a possible therapeutic target for modulation of renal vascular tone and resulting RVR. The direct role of elastin in the kidney, separate from its role in large and small artery hemodynamics, must also be addressed. This could be investigated through cell type–specific modulation of elastin expression.

DISCLOSURE

The author declared no competing interests.

REFERENCES

1. Le VP, Knutsen RH, Mecham RP, et al. Decreased aortic diameter and compliance precedes blood pressure increases in postnatal development of elastin-insufficient mice. *Am J Physiol Heart Circ Physiol*. 2011;301:H221–H229.
2. O'Rourke MF, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension*. 2005;46:200–204.
3. Owens EA, Li J, Reyes BAS, et al. Elastin insufficiency causes hypertension, structural defects and abnormal remodeling of renal vascular signaling. *Kidney Int*. 2017;92:1100–1118.
4. Osei-Owusu P, Knutsen RH, Kozel BA, et al. Altered reactivity of resistance vasculature contributes to hypertension in elastin insufficiency. *Am J Physiol Heart Circ Physiol*. 2014;306:H654–H666.
5. Guerin AP, Blacher J, Pannier B, et al. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation*. 2001;103:987–992.
6. Woodard T, Sigurdsson S, Gotal JD, et al. Mediation analysis of aortic stiffness and renal microvascular function. *J Am Soc Nephrol*. 2015;26:1181–1187.
7. Hashimoto J, Ito S. Aortic Blood Flow Reversal Determines Renal Function: Potential Explanation for Renal Dysfunction Caused by Aortic Stiffening in Hypertension. *Hypertension*. 2015;66:61–67.
8. Bensalah MZ, Bollache E, Kachenoura N, et al. Geometry is a major determinant of flow reversal in proximal aorta. *Am J Physiol Heart Circ Physiol*. 2014;306:H1408–H1416.
9. Smith ER, Tomlinson LA, Ford ML, et al. Elastin degradation is associated with progressive aortic stiffening and all-cause mortality in predialysis chronic kidney disease. *Hypertension*. 2012;59:973–978.

Going single but not solo with podocytes: potentials, limitations, and pitfalls of single-cell analysis

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Single-cell RNA-sequence (RNA-seq) is a widely used tool to study biological questions in single cells. The discussed study identified 92 genes being predominantly expressed in podocytes based on a 5-fold higher expression compared with endothelial and mesangial cells. In addition to technical pitfalls, the question that is discussed in this commentary is whether results of a single-cell RNAseq study are able to deliver expression data that truly characterize a podocyte.

Kidney International (2017) **92,** 1038–1041; http://dx.doi.org/10.1016/j.kint.2017.05.033

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Single-cell RNA sequence (RNA-seq) is a novel and powerful tool to study biological questions in single cells. It can be used to perform accurate quantitative transcriptome measurements in individual cells with a relatively small number of sequencing reads, and sequencing large numbers of single cells can recapitulate bulk transcriptome complexity.[1,2] Numerous methods have been published following one general workflow: after isolation of single cells, the cells are lysed, then

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reverse transcription is performed on the captured RNA, and the resulting cDNA is preamplified and used as a template for sequencing. Limitations of the method are a limited sensitivity to detect low abundance RNAs and "technical noise" due to the low amount of input material. Another technical bias is induced by the isolation techniques of single cells from tissues, and although the spatial context of a single cell is lost, the enzymatic procedures used might influence the cellular transcriptome and might influence cell viability.[2] Nevertheless, the method is a powerful tool to detect cell-to-cell variation that is masked by averaging artifacts in bulk measurements. Raser *et al.*[3] previously demonstrated using single-cell RNA-seq that the gene expression in single cells of the same type can be significantly different.[3] This is understandable because gene expression is very dynamic and cells can be in a different state when extracted. But can the method with the above-mentioned technical bias also help to identify unique genes of cell types because the identification of unique genes specific for a cell type is highly desirable to acquire insights into the cell's biological role and function? What defines a unique gene set apart from stable expression and what distinguishes these genes from housekeepers? Is it the response to stress factors, to physiologic stimuli, and to other cell types? Also, if this method is used to identify unique genes, how many different cells are necessary to define this particular gene set?

In this issue of the journal, Lu *et al.*[4] (2017) report on performing single-cell RNAseq on 20 mouse podocytes using a Fluidigm C1 system and comparing the identified genes with results from mesangial and endothelial cells isolated from mouse glomeruli. The authors claim that the cell-to-cell variation uncovered by the method can be used to identify unique genes because genes that are not expressed in all cells are likely dispensable for the cells structure and function. They isolated glomeruli from mice, dissociated them into single cells, made viability checks, and identified the podocyte cDNA samples using quantitative polymerase chain reaction analysis of the podocyte markers podocin, WT-1, and synaptopodin. Those samples were then used for RNAseq. They identified 92 genes being predominantly expressed in podocytes based on a 5-fold higher expression compared with the endothelial and mesangial cells (Figure 1). Among those genes were 27 genes that are already known to be involved in podocyte structure and/or function (e.g., nephrin, podocin, synaptopodin, WT-1, and vascular endothelial growth factor A). They also analyzed 37 novel genes by silencing and found that the reduced expression would either induce cytoskeletal injury in podocytes or a downregulation of CD2AP and synaptopodin.

The paper is not only interesting because it expands the list of genes that are essential for podocyte function, but

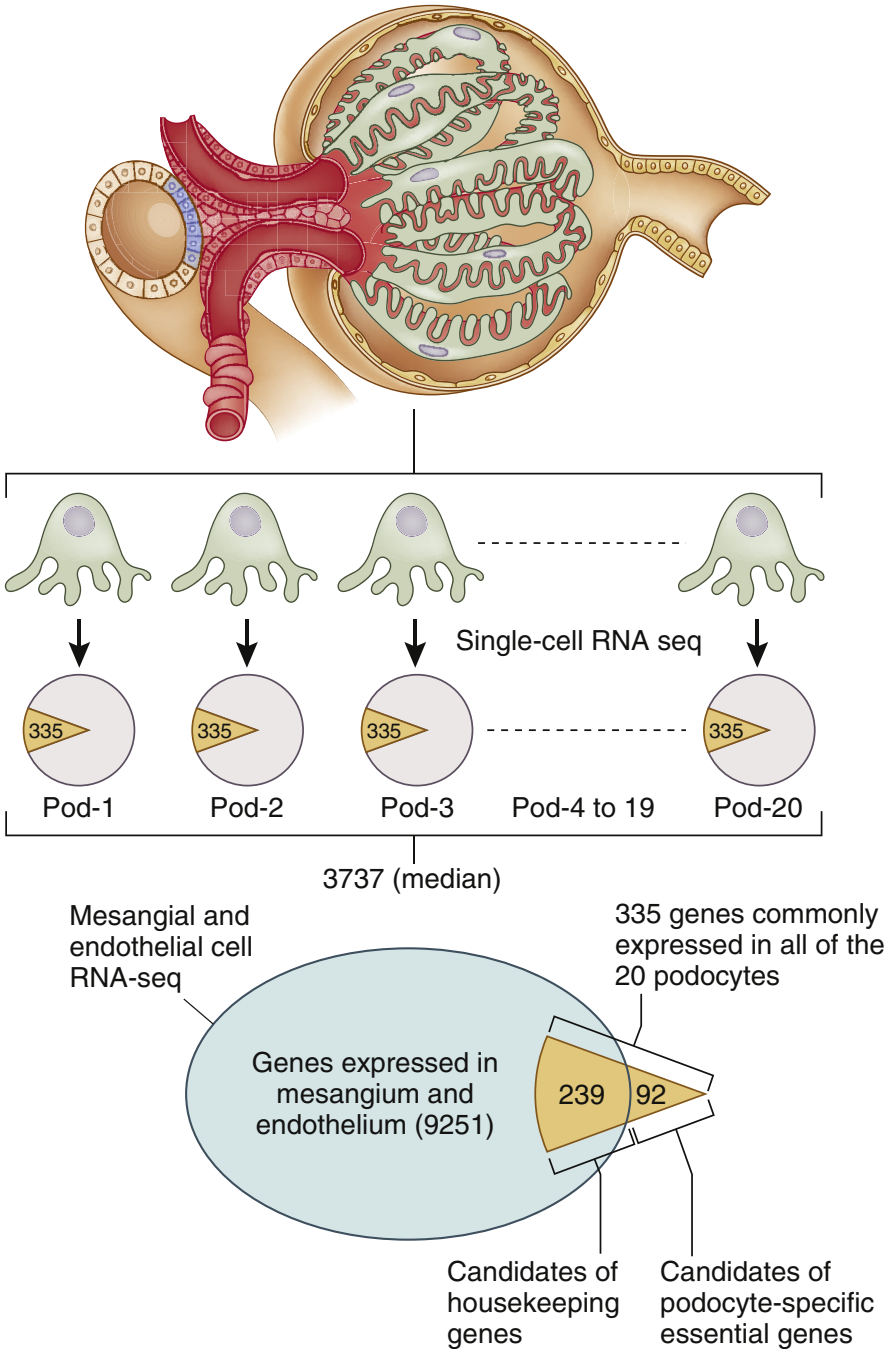

Figure 1 | Schematic work flow to identify "essential" podocyte transcripts.