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Capturing the *in vivo* molecular signature of the podocyte

Peter Y Chuang[1], **Jia Fu**[2], and **John C He**[1,3]

[1]Division of Nephrology, Department of Medicine, Mount Sinai School of Medicine, New York, New York

[2]Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

[3]Department of Medicine, James J Peters Veterans Affairs Medical Center, Bronx, New York

Over the past 15 years, the number of podocyte-related publications per year has increased exponentially (Fig 1). This trend coincided with the identification of nephrin[1], a discovery that brought to light the pivotal role of the podocyte in glomerular filtration; and the availability of a conditionally immortalized podocyte cell line[2], making podocytes accessible for studies in cell culture systems. The availability of genetic tools that allow podocyte-specific manipulation of gene expression in animals (i.e. podocyte-specific expression of Cre recombinase and reverse tetracycline transactivator) further hastened the pace of podocyte research. In spite of these innovations, there is still a critical gap in our knowledge as well as the ability to examine the molecular attributes of the podocyte in health and disease.

The podocyte is a terminally differentiated, highly specialized epithelial cell. It is clear that podocytes maintained *ex vivo* in cell culture systems do not completely recapitulate the morphologic and molecular phenotype of podocytes *in vivo*. Furthermore, in most disease models it is impossible to replicate the *in vivo* disease milieu under *ex vivo* culture conditions. Since *ex vivo* cultured podocytes are not adequate substitutes for *in vivo* podocytes, many have attempted to isolate primary podocytes from animals. However, none of the existing protocols for podocyte isolation provide sufficient quality and yield to permit types of omics-level analysis other than transcriptome profiling. This has been a major quandary in the field of podocyte research.

Intimate association of the three glomerular cell types—podocytes, mesangial cells, and endothelial cells—makes the isolation of podocytes from biological samples difficult. Since glomerular volume in man and mice accounts for less than 5% of the total renal mass and podocytes are only present in the glomerulus, glomerular extracts are enriched in podocytes relative to the rest of the kidney. Glomeruli can be isolated with >95% purity from man and rats using sieving techniques[3] and from mice by perfusion of magnetic particles followed by magnetic isolation[4, 5]. While some have used glomerular extracts to obtain a first approximation of the podocyte-specific attributes, the joint presence of mesangial and endothelial cells with podocytes in the glomerulus precludes definitive characterization of podocyte-specific features. To overcome this, attempts have been made to isolate primary podocytes from glomerular extract after enzymatic digestion to dissociate glomeruli into a single cell suspension. Following this, podocytes are distinguished from non-podocytes

either by using antibodies against podocyte-specific antigens, such as nephrin[6] and podocalyxin[7], or through the use of fluorescent markers that are expressed exclusively in podocytes of transgenic mice[8]. Podocytes are separated from non-podocyte cells using fluorescence[7, 8] or magnetic[6]-activated cell sorting techniques. A major limitation of these approaches, however, is the low yield of extraction.

In this issue, Boerries *et al.*[9] described a modified podocyte extraction procedure. This protocol improved the glomerular yield by approximately 2-to-3 fold compared to the original protocol of glomerular isolation by perfusion of magnetic particles[5]. To distinguish between podocyte and non-podocyte glomerular cells, Boerries *et al.* generated a bitransgenic line of mice with podocyte-specific expression of a green fluorescence protein by breeding a Cre-reporter strain of mice (*mT/mG*) to mice with podocyte-specific expression of Cre-recombinase (*hNPHS2Cre*). Glomeruli were isolated from bitransgenic mice (*mT/mG;hNPHS2Cre*) using perfusion of magnetic particles and then were dissociated into a single cell suspension prior to fluorescence-activated sorting of podocytes. The yield of extraction was greater than 5×10^5 podocytes per mouse, which was sufficient for transcriptome profiling as well as quantitative proteomic analysis when freshly isolated podocytes from two mice were pooled. Bioinformatic analyses of the transcriptomic and proteomic datasets revealed that gene regulation programs pertaining to endoplasmic reticulum, ubiquitination, cytoskeleton, nuclear elements and mitochondria, peroxisome and protein transport, and cell junction were enriched in podocytes.

This study by Boerries *et al.* contributes to the study of podocyte biology in several important ways. Their modified isolation protocol improved on the yield of podocyte extraction, which allowed quantitative proteomic analysis to be performed on isolated podocytes. Comparison of the transcriptome and proteome revealed a lack of perfect correlation between the protein and transcript levels for some genes, which suggests that these genes are regulated at the post-transcriptional level. Since the regulation of gene expression is not fully represented by the transcriptome alone, proteomic data provides another layer of information. Although the transcriptomes of isolated podocytes have been published by others, none of those studies have juxtaposed their results with prior publications. Boerries *et al.* compared the transcriptome of isolated podocytes to other publically available transcriptomes of podocytes from different sources: adult mice, developing mouse embryo, and cultured podocytes. Boerries *et al.* also performed additional bioinformatics analyses of the transcriptomic and proteomic datasets. The combined datasets predict several biological functions, not previously described, that could be important for the maintenance of the podocyte.

While the approach taken by Boerries et al. to isolate podocytes is an improvement from prior protocols, it remains imperfect. All existing podocyte isolation protocols require enzymatic digestion at 37°C to separate glomeruli from surrounding non-glomerular structures and to dissociate podocytes from non-podocyte glomerular cells. Mechanical disruption and enzymatic digestion at 37°C are likely to alter the molecular signature of the podocytes during the isolation process. Unfortunately, no alternative approach exists to avoid enzymatic digestion at 37°C. Another point to consider is the use of transgenic expression of a fluorescent tag for podocyte sorting as opposed to antibody-based sorting.

Genetic tagging of podocytes has the disadvantage of time and cost associated with backcrossing and breeding when studying models of kidney disease where the disease is due to a genetic alteration (i.e. *db/db* model of diabetes, Tg26 model of HIV associated nephropathy, or CD2AP null mice). However, this will need to be balanced with the higher yield and purity of the transgenic approaches compared to antibody-based methods. Finally, we also need to be cognizant of the fact that although bioinformatic-based analyses by Boerries *et al.* yielded predictions about biological processes that are enriched in the podocytes, these predictions will require experimental confirmation before they can be considered biologically valid and functionally important.

This study by Boerries *et al.* is the first to characterize the transcriptome and proteome of podocytes using cells that were procured in a way that was least likely to perturb their *in vivo* molecular signature. These datasets are valuable resources for the podocyte research community and can be used to establish the core transcriptome and proteome of the podocyte. Application of the podocyte extraction protocol to disease models followed by downstream characterization of the proteome, transcriptome, and epigenome will facilitate the progress of research and further our understanding of podocytes in kidney health and disease.

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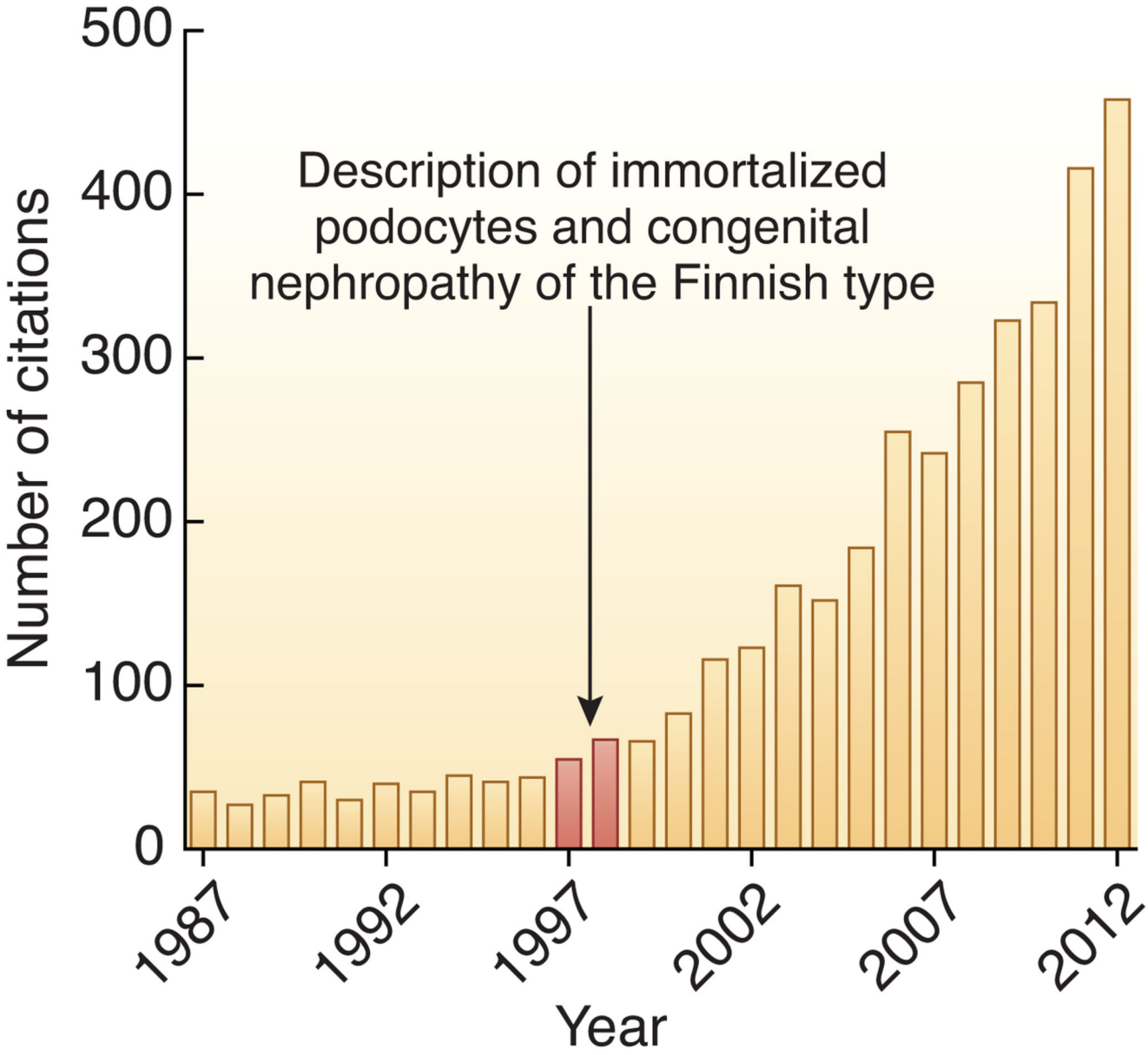

Figure 1.
Number of PubMed citations for the term *podocyte* from 1987 to 2012. Conditionally immortalized murine podocytes and congenital nephropathy of the Finnish type were reported in 1997 and 1998, respectively.