Applications of Gene Therapy Strategies for Podoycte Function and CKD

Sepehr Khavari*

* – corresponding author, sepehrkhavari13@gmail.com

Abstract

Podocyte injury is a significant factor in the pathogenesis of chronic kidney disease, which has become increasingly abundant on a global scale. Pathological insults - including genetic mutations and oxidative stress - can disrupt the integrity of podocytes, specialized epithelial cells that are components in the glomerular filtration barrier. Potential therapeutic strategies to target podocyte dysfunction involve the application of gene therapies as well as a greater understanding of the mechanisms of podocyte injury. Showing promise in recent clinical studies, gene therapies rely on AAV vectors to deliver the treatment to the correct target with high efficacy. In addition, with the rise of FDA approvals for gene therapy treatment in humans, therapies such as PS-001 offer promising results for treating genetic kidney disorders. Beyond the therapy itself, new advances in delivery platforms can aid in minimizing systemic toxicity. However, the limited regenerative capabilities of podocytes and inadvertent side effects must still be addressed in both the creation and delivery of new treatments.

Introduction

Because podocyte injury is notable in its role in the pathogenesis of kidney disease, treatments to address the progressive loss of kidney function in chronic kidney disease (CKD) can target podocytes: these specialized epithelial cells maintain kidney function by forming a layer of the glomerular filtration barrier (Finch et al.). The dysfunction of podocytes is a notable driver of CKD progression due to the limited capacity of podocytes to regenerate, which means that damage to podocytes has significant ramifications. Mechanisms of podocyte injury have recently been shown to involve genetic mutations and oxidative stress as integral components. However, treatments for kidney diseases that correlate with podocyte dysfunction are being developed (X. Li et al.).

One promising approach seeks to influence podocyte injury on the molecular level: allowing for the modulation of pathways implicit in podocyte function, gene therapy promotes protective gene expression while simultaneously hindering harmful proteins from being synthesized (Ertl). Beyond the creation of pharmaceuticals to administer to patients, current advancements are being developed to increase the efficacy of delivering therapeutic genes to podocytes. Although promising, inadvertent effects such as off-target delivery and chronic usage must still be addressed.

History

Podocytes—specialized epithelial cells—primarily function to maintain the health of the kidneys in the body by formulating an outside layer of the glomerular filtration barrier; this is crucial for both the regulation of blood filtration and the prevention of the degradation of essential proteins—including albumin—into the urine. Podocytes have a structure composed of interdigitating foot processes that interlock to create a layer similar to mesh, which allocates the selective permeability for this barrier. This structure is supported by a complex actin cytoskeleton that is responsible for the maintenance of podocyte integrity. This podocyte layer ensures the retention of larger molecules, such as proteins, in the bloodstream and prevents proteins's passage into the urine (Mou et al.).

Studies have highlighted podocytes' terminal differentiation and limited regenerative capacity, crucial functions that underscore the importance of the podocyte's ability to maintain the integrity of the filtration barrier. As a result, podocytes are particularly vulnerable to certain kidney diseases: minimal change disease (MCD), diabetic nephropathy, and focal segmental glomerulosclerosis (FSGS) are notable examples. With podocyte depletion leading to the filtration barrier's breakdown, the link between CKD progression and podocyte injury has become well-established with subsequent kidney damage and proteinuria. (Jiang et al.)

Early podocyte research and mechanistic studies have identified pathways that correlate with podocyte dysfunction. Hyperglycemia in diabetes is one such pathway that initiates cascades potentially culminating in cellular injury, which is a result of the upregulation of the prorenin receptor (PRR) in podocytes (Ye et al.). This upregulation is responsible for the activation of pathways that are pro-fibrotic and pro-inflammatory that exacerbate podocyte damage. Moreover, free fatty acids can induce mitochondrial dysfunction and apoptosis, a ramification of lipid accumulation, and oxidative stress (Wang et al.). The integrity of podocytes is a requisite for the maintenance of normal kidney function. Thus, when this function begins to wane from these pathways, the likelihood of the development and progression of CKD increases, which makes it important to develop necessary treatments.

These findings have led to an emphasis on translational research that applies knowledge of these mechanisms to therapeutic strategies. For instance, extracellular vesicles from podocytes have been identified as biomarkers for early injury in the kidneys. Li et al. Including microRNAs and pathways that serve to regulate function and cellular survival, gene expression studies have found potential therapeutic targets. (Yahya and Elsayed).

Initial efforts have identified critical genes—such as nephrin and podocin—that are critical for the maintenance of the filtration barrier (Solovev). Subsequent research has been made to develop gene therapy strategies that aim to restore function in designated protein expression pathways (Zhang).

Mechanisms of Podocyte Injury

Podocyte injury in chronic kidney disease (CKD) can be exacerbated by oxidative stress, a primary contributor through the accumulation of reactive oxygen species (ROS). Elevated glucose levels in diabetic nephropathy (DN) lead to increased ROS production, which damages macromolecules and other cellular components. (Fang et al.). Another consequence of oxidative stress is mitochondrial dysfunction, which is linked to mitochondrial fission and the subsequent ROS production to apoptosis in podocytes (Zhong et al.). Furthermore, the depletion of podocytes is another result of apoptosis induced by oxidative stress; this has been linked to characteristic proteinuria observed in CKD. Makhammajanov et al.).

Another pathway that is a notable cause for the dysfunction of podocytes is inflammation; this is particularly through cytokines like TNF-α that disrupt the glomerular filtration barrier's integrity. The effect is increased permeability and proteinuria (Wang et al.). The NF-κB signaling pathway further damages podocytes by upregulating pro-inflammatory cytokines as it is activated in response to inflammatory stimuli (Ma et al.). However, this inflammatory response occurs in local contexts—like non-alcoholic steatohepatitis (NASH)—and not only in systemic conditions (Li et al.).

Hereditary conditions that involve podocyte dysfunction and detachment, such as Fabry disease, are the result of genetic mutations. Specifically, mutations in the GLA gene lead to the accumulation of glycosphingolipids. This leads to the promotion of podocyte apoptosis and disruption of cellular functions (Furia et al.). Recent findings have highlighted that these mutations in GLA correlate with autophagy and apoptosis (P. Li et al.).

The use of gene therapy to address podocyte dysfunction represents a strong candidate: for conditions where genetic mutations or deficiencies directly impact kidney function, this method is particularly relevant. For example, Glis2 and other long noncoding RNAs have been implicated as regulatory elements in modulating mitochondrial dysfunction and apoptosis in podocytes. In a recent study, the overexpression of Glis2 has been correlated with the minimization of podocyte apoptosis that is induced by hyperglycemia. It acts as a competing endogenous RNA (ceRNA) for miR-328-5 to negatively regulate notable protective genes like Sirt1 (C. Zhang).

Contrary to the overexpression of some genes, silencing or methylation of detrimental genes offers another therapeutic option. For instance, the E3 ubiquitin ligase Trim63 promotes podocyte injury by inhibiting the expression of PPARα (Peroxisome Proliferator-Activated Receptor Alpha). Therefore, strategies could reduce apoptosis and proteinuria by methylating or silencing Trim63 to restore PPARα activity (Sylwia Słuczanowska-Głabowska et al.).

Mitochondrial dysfunction and autophagy are two additional mechanisms that could be effectively addressed with gene therapeutics. The enhancement of the expression of Mfn2—a mitochondrial fusion protein—or Drp1—a silencing fission protein—could improve mitochondrial function and thereby reduce apoptosis in podocytes (Y. Li et al.). Contrarily, promoting autophagy through gene therapy by targeting certain genes that express autophagy, including Beclin-1 or LC3, could offer protection for podocytes through the clearance of damaged or misfolded organelles and proteins (Tseng et al.).

However, there are complications that arise from the current understanding of the mechanisms of podocyte injury and consequently the formulation of effective therapies to address these mechanisms. One issue is that podocytes hold a limited regenerative capacity: irreversible kidney damage is more likely as podocytes are post-mitotic cells and have a reduced ability to heal once damaged (Montenegro et al.).

Gene Therapy Implementation

With the rise of FDA approval for genetic therapies in clinical applications, gene therapies for podocyte dysfunction and chronic kidney disease have risen in popularity. One such example is

PS-001, which represents an application that utilizes AAV (Adeno-Associated Virus) gene therapy to provide the NPHS2 gene—encoding podocin—directly to the podocytes (S. Illingworth et al.). The therapy itself is designated for steroid-resistant nephrotic syndrome, which is induced by mutations in the NPHS2 gene. PS-001 demonstrated significant efficacy in preclinical studies involving murine models as it demonstrated a marked reduction in proteinuria: the mean albumin-to-creatinine ratio (ACR) decreased from 6049 ± 2803 to 192 ± 51. Furthermore, there was a strong decrease in glomerulosclerosis as the percentage of affected glomeruli decreased from 81% ± 19 to 18.75% ± 12. A supporting study that utilized pig models demonstrated transgene mRNA expression of 89% ± 11% for kidney glomeruli, and no off-target effects were detected (S. Illingworth et al.). This efficacy has led PS-001 to potentially become the first podocyte-targeted gene therapy to enter clinical development.

Alport syndrome is a genetic disorder characterized by mutations in collagen IV genes (COL4A3/A4/A5)—genes that are responsible for the maintenance of the glomerular basement membrane—and is thus a target for gene therapy. Similar to PS-001, the AAV-based gene therapy strategy delivers the genes encoding collagen IV components directly to podocytes. In preclinical studies in animal models, AAV-mediated gene delivery can restore collagen IV expression and improve kidney function (Y. Zhao et al.). A dual AAV system has been proposed to overcome gene load limitations that are inherent in AAV vectors, which allows for the effective delivery of larger genes (van Lieshout et al.).

Adriamycin-induced nephropathy—a model for focal segmental glomerulosclerosis (FSGS)—is an injury that can be protected by the overexpression of Asparagine Endopeptidase (AEP) in podocytes. Studies that utilized mouse models saw a reduction in podocyte loss and an overall improvement in kidney function stemming from the conditional overexpression of AEP (Y. Qiu et al.). A therapeutic target for protecting podocytes from injury, AEP could serve as a novel treatment. However, the broader implications of applicability to different variations of CKD and the clinical efficacy of this approach must be explored.

Gene Therapy Delivery Systems

More precise and effective strategies for gene therapies that target podocytes in chronic kidney disease (CKD) are enhanced by recent innovations in drug delivery systems. The Dex/PFP@LIPs-BMS-α nano delivery system is one such advancement, specifically designated for immune-associated nephropathy. This approach combines liposomes, modified with BMS-470539 (BMS-α), that act as targeting ligands with dexamethasone (Dex) and perfluoropentane (PFP). A potent glucocorticoid, Dex minimizes systemic side effects by being encapsulated within liposomes to reduce immune responses and inflammation (Z. Wen et al.). With the target site being addressed with ultrasound-targeted microbubble destruction (UTMD), the inclusion of perfluoropentane allows for controlled release of Dex. By targeting the

melanocortin-1 receptor (MC-1R) expressed on podocytes, the use of BMS-470539 increases the specificity of the system; this ensures that Dex's therapeutic effects are localized to these epithelial cells. Significant improvements were seen with the usage of this system that demonstrated lower serum creatinine levels and a reduction in proteinuria in preclinical studies, importantly without significant side effects (Z. Wen et al.). Therefore, the Dex/PFP@LIPs-BMS-α nano delivery system has shown potential to be a treatment of high efficacy for immune-associated nephropathy.

Engineered to encapsulate therapeutics such as plasmids or small molecules, nanocapsules target podocytes by penetrating the glomerular filtration barrier. The vessels are composed of biocompatible materials like chitosan with dimensions of approximately 60 nm; the ability to control the release of encapsulated drugs and protect the drugs from degradation before reaching the target site is facilitated by the multilayer structure of the nanocapsules (Chesneau et al.). A potential avenue that utilizes this particular delivery system is through gene therapy applications to minimize the expression of harmful proteins that are associated with podocyte diseases, such as c-mip.

Utilizing phospholipid nanoparticles encapsulating celastrol—a natural compound known for its anti-inflammatory and protective effects on kidney cells—the PC-PLN (Peptide Coupled Celastrol-Phospholipid Nanoparticles) delivery system is a drug delivery system used in CKD treatment that targets glomerular endothelial cells. Particularly during inflammatory conditions, the nanoparticles target the glomerular region of the kidney through modification with specific peptides (Y. Liu). The effective delivery of celastrol directly to the damaged cells is ensured by PC-PLN, along with improved accumulation of nanoparticles at the site of injury. The combination of decreased levels of inflammatory markers, such as VCAM-1 and TNF-α, and increased levels of protective factors, like endothelial nitric oxide synthase (eNOS) and nitric oxide (NO), was seen in models that applied PC-PLN. These studies also exhibited reduced glomerular damage in models of immunoglobulin A nephropathy (IgAN) and chronic progressive nephropathy (CPN) (Q. Wu et al.). The additional reduced systemic toxicity profile for celastrol compared to traditional administration methods allows this drug delivery system to have high versatility among a large variety of conditions.

Similarly, for diabetic kidney disease (DKD), dual-target nanoparticles are designed to attach to multiple sites to address multiple mechanisms of the disease simultaneously. This delivery system allows for inhibitors, such as angiotensin-converting enzyme (ACE) or receptor for advanced glycation end products (RAGE), to be delivered concurrently to different sites like the glomerular endothelium and podocytes (X. Chen et al.).

Albumin-based nanoparticles are a biocompatible drug delivery system that utilizes human serum albumin (HSA) as a carrier. The nanoparticles are advantageous due to their ability to bind

multiple pharmaceuticals and their inherent targeting properties. Facilitating the endocytosis of albumin and allowing for targeted delivery of encapsulated drugs directly to the kidney is allocated by albumin-based nanoparticles that exploit the neonatal Fc receptor (FcRn) present on podocytes and other kidney cells (B. Wu). Glucocorticoids, such as methylprednisolone, benefit by being able to help mitigate systemic side effects commonly associated with high doses of these medications by being delivered to glomerular podocytes. Reduction of proteinuria and improvement in models of kidney disease were seen with the use of albumin-based nanoparticles. This highlights the efficacy of this delivery system as a safe therapeutic strategy.

Issues with Gene Therapy for Podocytes and CKD

While this treatment option holds promise, there is an inherent risk of the inadvertent interaction of non-target genes or tissues with therapeutic genes or vectors (C. Guo et al.). Although commonly utilized for their relatively minimal immunogenicity and safety profile, adeno-associated virus (AAV) vectors are a traditional delivery system that is not exempt from this risk. Thus, there remains a possibility for integration with sites within the host genome that are unintended to be treated, possibly triggering adverse health effects (Q. Fu et al.). This underscores the importance of designing methods to prevent inadvertent reactions.

Potential toxicity through the administration of large doses of viral vectors is another issue that must be addressed: high doses can provoke immune responses that may damage podocytes or other renal structures and minimize the possibility of repeated dosing regimens (R. Khan et al.). In chronic conditions such as CKD, this immune activation can be problematic where sustained treatment may be a priority. Beyond potency from excessive admonition, systemic toxicity will be an issue to be approached for drug delivery systems, especially those that utilize glucocorticoids. Including metabolic disturbances and an increased risk of infections, traditional glucocorticoid therapies have had inadvertent ramifications (Frodlund et al.).

The ability of the glomerular basement membrane (GBM) to have selective permeability also makes it difficult to have efficient delivery to podocytes (Peek and Wilson). Without affecting other renal tissues, gene therapy delivery must be improved to overcome the GBM.

Since these advancements in gene therapy are relatively recent, longitudinal studies must be emphasized to assess the possible chronic effects of the administration of gene therapy. For instance, in animal models, PS-001 did not demonstrate any adverse effects (Illingworth et al.). Toxicological assessments in preclinical studies must be utilized; they are essential for evaluating the safety profile of gene therapies; continuous monitoring will be necessary to move from preclinical models to human trials (Kohn et al.).

Currently, there is also a limitation to the efficacy of gene therapy approaches for podocytes and their related diseases, as there is difficulty in achieving long-term expression of the therapeutic gene (Khorkova et al.). However, particularly in the presence of an active immune response, viral vectors will not be able to promote targeted gene expression over extended behavior. In order to maintain therapeutic levels of gene expression, more stable vectors must be developed to deter frequent re-administration.

Beyond potency from excessive admonition, systemic toxicity will be an issue to be approached for drug delivery systems, especially those that utilize glucocorticoids. Including metabolic disturbances and an increased risk of infections, traditional glucocorticoid therapies have had inadvertent ramifications (N. Patel et al.).

The ability to monitor treatment efficacy is currently limited by the lack of visual guidance, as most drug delivery systems do not incorporate imaging capabilities. This deters the ability to monitor the efficacy of therapeutic strategies. To integrate ultrasound or photoacoustic imaging, further development before wider adoption in clinical settings is needed. As a result, emerging systems have arisen, yet these approaches are still in their infancy. This need for imaging is underscored by the concern for the durability of gene delivery systems, as quick clearance of the drug before it reaches the target site results from rapid burst release during circulation (Ezike et al.).

Regulatory and ethical considerations may hamper gene therapy's translation from laboratory research to clinical application. Many promising gene therapies are still in their research phase and have yet to move to clinical practice. To establish the safety of these therapies to approval agencies, extensive preclinical and clinical testing will be required, and while necessary, these regulatory requirements will slow down the process of bringing new treatments to patients (Adair et al.).

Conclusion

As a driver of conditions that are influenced by podocyte dysfunction, podocyte injury has emerged as a promising target for gene therapy. This has been due to new understandings of the molecular mechanisms that govern the function of pathways for podocytes. New advancements in this form of therapeutic strategy aim to both preserve and restore podocyte function. With the recent surge of FDA approval for the administration of gene therapy into patients, therapeutics like PS-001 and nanoparticle-based delivery systems can have the potential to reduce proteinuria and mitigate the progression of diseases such as CKD. However, as with all treatments that seek to transition from preclinical studies to clinical practice, regulatory and safety hurdles will have to be addressed, including potential chronic side effects and immune reactions.

References

Adair, Jennifer E., Lindsay Androski, Lois Bayigga, Deus Bazira, Eugene Brandon, Lynda Dee, Steven Deeks, et al. “Towards Access for All: 1st Working Group Report for the Global Gene Therapy Initiative (GGTI).” *Gene Therapy* 30, no. 3 (April 2023): 216–21. https://doi.org/10.1038/s41434-021-00284-4.

Arthur, Gertrude, Jeffrey L. Osborn, and Frédérique B. Yiannikouris. “(Pro)Renin Receptor in the Kidney: Function and Significance.” *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 320, no. 4 (April 1, 2021): R377–83. https://doi.org/10.1152/ajpregu.00259.2020.

Audzeyenka, Irena, Agnieszka Bierżyńska, and Abigail C Lay. “Podocyte Bioenergetics in the Development of Diabetic Nephropathy: The Role of Mitochondria.” *Endocrinology* 163, no. 1 (January 1, 2022): bqab234. https://doi.org/10.1210/endocr/bqab234.

Banu, Khadija, Qisheng Lin, John M. Basgen, Marina Planoutene, Chengguo Wei, Anand C. Reghuvaran, Xuefei Tian, et al. “AMPK Mediates Regulation of Glomerular Volume and Podocyte Survival.” *JCI Insight* 6, no. 19 (October 8, 2021): e150004. https://doi.org/10.1172/jci.insight.150004.

Bellur, Shubha S., Fanny Lepeytre, Olga Vorobyeva, Stéphan Troyanov, H. Terence Cook, Ian S. D. Roberts, and International IgA Nephropathy Working Group. “Evidence from the Oxford Classification Cohort Supports the Clinical Value of Subclassification of Focal Segmental Glomerulosclerosis in IgA Nephropathy.” *Kidney International* 91, no. 1 (January 2017): 235–43. https://doi.org/10.1016/j.kint.2016.09.029.

Bryer, Joshua S., and Katalin Susztak. “Screening Drugs for Kidney Disease: Targeting the Podocyte.” *Cell Chemical Biology* 25, no. 2 (February 15, 2018): 126–27. https://doi.org/10.1016/j.chembiol.2018.01.018.

Chen, Qiyan, Chao Xie, Kaiyue Tang, Mujin Luo, Zhe Zhang, Yabin Jin, Youhua Liu, Lili Zhou, and Yaozhong Kong. “The E3 Ligase Trim63 Promotes Podocyte Injury and Proteinuria by Targeting PPARα to Inhibit Fatty Acid Oxidation.” *Free Radical Biology and Medicine* 209 (November 2023): 40–54. https://doi.org/10.1016/j.freeradbiomed.2023.09.039.

Chen, Xian, Wenni Dai, Hao Li, Zhe Yan, Zhiwen Liu, and Liyu He. “Targeted Drug Delivery Strategy: A Bridge to the Therapy of Diabetic Kidney Disease.” *Drug Delivery* 30, no. 1 (December 31, 2023): 2160518. https://doi.org/10.1080/10717544.2022.2160518.

Chesneau, Cléa, Laura Larue, and Sabrina Belbekhouche. “Design of Tailor-Made Biopolymer-Based Capsules for Biological Application by Combining Porous Particles and Polysaccharide Assembly.” *Pharmaceutics* 15, no. 6 (June 2023): 1718. https://doi.org/10.3390/pharmaceutics15061718.

Clark, Abbe R., Jamie Marshall, Yiming Zhou, Monica S. Montesinos, Haiqi Chen, Lan Nguyen, Fei Chen, and Anna Greka. “Single-Cell Transcriptomics Reveal Disrupted Kidney Filter Cell-Cell Interactions after Early and Selective Podocyte Injury.” *The American Journal of Pathology* 192, no. 2 (February 2022): 281–94. https://doi.org/10.1016/j.ajpath.2021.11.004.

Daehn, Ilse S., and Jeremy S. Duffield. “The Glomerular Filtration Barrier: A Structural Target for Novel Kidney Therapies.” *Nature Reviews Drug Discovery* 20, no. 10 (October 2021): 770–88. https://doi.org/10.1038/s41573-021-00242-0.

Don, Paththamperuma Arachchige, and Jeral Chathura Madushanka P. “The Study of Structural and Mechanistic Features of Hsp70/CHIP-Driven Protein Quality Control.” Miami University, 2023. https://etd.ohiolink.edu/acprod/odb_etd/etd/r/1501/10?clear=10&p10_accession_num=miami167155209682274.

Ertl, Hildegund C. J. “Mitigating Serious Adverse Events in Gene Therapy with AAV Vectors: Vector Dose and Immunosuppression.” *Drugs* 83, no. 4 (March 1, 2023): 287–98. https://doi.org/10.1007/s40265-023-01836-1.

Ezike, Tobechukwu Christian, Ugochukwu Solomon Okpala, Ufedo Lovet Onoja, Chinenye Princess Nwike, Emmanuel Chimeh Ezeako, Osinachi Juliet Okpara, Charles Chinkwere Okoroafor, et al. “Advances in Drug Delivery Systems, Challenges and Future Directions.” *Heliyon* 9, no. 6 (June 1, 2023). https://doi.org/10.1016/j.heliyon.2023.e17488.

Fan, Kui, Li Zeng, Jing Guo, Shuqin Xie, Yuan Yu, Jianwei Chen, Jin Cao, et al. “Visualized Podocyte-Targeting and Focused Ultrasound Responsive Glucocorticoid Nano-Delivery System against Immune-Associated Nephropathy without Glucocorticoid Side Effect.” *Theranostics* 11, no. 6 (2021): 2670–90. https://doi.org/10.7150/thno.53083.

Feng, Di, Mukesh Kumar, Jan Muntel, Susan B. Gurley, Gabriel Birrane, Isaac E. Stillman, Lai Ding, et al. “Phosphorylation of ACTN4 Leads to Podocyte Vulnerability and Proteinuric Glomerulosclerosis.” *Journal of the American Society of Nephrology: JASN* 31, no. 7 (July 2020): 1479–95. https://doi.org/10.1681/ASN.2019101032.

Finch, Natalie C., Chris R. Neal, Gavin I. Welsh, Rebecca R. Foster, and Simon C. Satchell. “The Unique Structural and Functional Characteristics of Glomerular Endothelial Cell

Fenestrations and Their Potential as a Therapeutic Target in Kidney Disease." *American Journal of Physiology-Renal Physiology* 325, no. 4 (October 2023): F465–78. https://doi.org/10.1152/ajprenal.00036.2023.

Fones, Helen N., Daniel P. Bebber, Thomas M. Chaloner, William T. Kay, Gero Steinberg, and Sarah J. Gurr. "Threats to Global Food Security from Emerging Fungal and Oomycete Crop Pathogens." *Nature Food* 1, no. 6 (June 8, 2020): 332–42. https://doi.org/10.1038/s43016-020-0075-0.

Frodlund, Martina, Andreas Jönsen, Lauren Remkus, Gunilla Telg, Fabian Söderdahl, and Dag Leonard. "Glucocorticoid Treatment in SLE Is Associated with Infections, Comorbidities and Mortality—a National Cohort Study." *Rheumatology* 63, no. 4 (April 1, 2024): 1104–12. https://doi.org/10.1093/rheumatology/kead348.

Fu, Qiang, Ashli Polanco, Yong Suk Lee, and Seongkyu Yoon. "Critical Challenges and Advances in Recombinant Adeno-Associated Virus (rAAV) Biomanufacturing." *Biotechnology and Bioengineering* 120, no. 9 (2023): 2601–21. https://doi.org/10.1002/bit.28412.

Furia, Alessandro, Raffaello Ditaranto, Elena Biagini, Vanda Parisi, Alex Incensi, Sara Parisini, Rocco Liguori, and Vincenzo Donadio. "Fabry Disease in W162C Mutation: A Case Report of Two Patients and a Review of Literature." *BMC Neurology* 24, no. 1 (April 5, 2024): 113. https://doi.org/10.1186/s12883-024-03540-3.

Gao, Shaohui, Yi Li, Tangsong Zhu, Zhaochen Guo, Yang Hu, Yong Wang, Beiduo Lv, et al. "Rs-TAC PDC, a Peptide Drug-Conjugate, for Targeted Delivery of Tacrolimus and Sericin Alleviates Podocyte Injury in Diabetic Nephropathy." *Nano Today* 57 (August 2024): 102385. https://doi.org/10.1016/j.nantod.2024.102385.

Gong, Minmin, Yujin Guo, Hui Dong, Fan Wu, Qiongyao He, Jing Gong, and Fuer Lu. "Modified Hu-Lu-Ba-Wan Protects Diabetic Glomerular Podocytes via Promoting PKM2-Mediated Mitochondrial Dynamic Homeostasis." *Phytomedicine* 123 (January 2024): 155247. https://doi.org/10.1016/j.phymed.2023.155247.

Guo, Congting, Xiaoteng Ma, Fei Gao, and Yuxuan Guo. "Off-Target Effects in CRISPR/Cas9 Gene Editing." *Frontiers in Bioengineering and Biotechnology* 11 (March 9, 2023). https://doi.org/10.3389/fbioe.2023.1143157.

Hirst, Jennifer A., Nathan Hill, Chris A. O'Callaghan, Daniel Lasserson, Richard J. McManus, Emma Ogburn, José M. Ordóñez Mena, et al. "Prevalence of Chronic Kidney Disease in the Community Using Data from OxRen: A UK Population-Based Cohort Study." *The British*

Journal of General Practice: The Journal of the Royal College of General Practitioners 70, no. 693 (April 2020): e285–93. https://doi.org/10.3399/bjgp20X708245.

https://doi.org/10.1016/j.ymthe.2023.09.003.

Illingworth, Sam, Kritima Gurung, Valeryia Kuzmuk, Hannah Wood, Rowan Asfahani, Alice Gennari, Wen Ding, et al. "#1118 Direct Delivery of Novel Gene Therapy to Podocytes Enables Pathway to Clinical Translation for the Treatment of Glomerular Diseases." *Nephrology Dialysis Transplantation* 39, no. Supplement_1 (May 1, 2024): gfae069-0032–1118. https://doi.org/10.1093/ndt/gfae069.032.

Jiang, Hong, Zhirang Shen, Jing Zhuang, Chen Lu, Yue Qu, Chengren Xu, Shufen Yang, and Xuefei Tian. "Understanding the Podocyte Immune Responses in Proteinuric Kidney Diseases: From Pathogenesis to Therapy." *Frontiers in Immunology* 14 (January 15, 2024). https://doi.org/10.3389/fimmu.2023.1335936.

Jiang, Hong, Zhirang Shen, Jing Zhuang, Chen Lu, Yue Qu, Chengren Xu, Shufen Yang, and Xuefei Tian. "Understanding the Podocyte Immune Responses in Proteinuric Kidney Diseases: From Pathogenesis to Therapy." *Frontiers in Immunology* 14 (2023): 1335936. https://doi.org/10.3389/fimmu.2023.1335936.

Kang, Jeong Suk, Nam-Jun Cho, Seong Woo Lee, Jeong Geon Lee, Ji-Hye Lee, Jawoon Yi, Min Sun Choi, et al. "RIPK3 Causes Mitochondrial Dysfunction and Albuminuria in Diabetic Podocytopathy through PGAM5-Drp1 Signaling." *Metabolism* 159 (October 2024): 155982. https://doi.org/10.1016/j.metabol.2024.155982.

Khorkova, Olga, Jack Stahl, Aswathy Joji, Claude-Henry Volmar, and Claes Wahlestedt. "Amplifying Gene Expression with RNA-Targeted Therapeutics." *Nature Reviews Drug Discovery* 22, no. 7 (July 2023): 539–61. https://doi.org/10.1038/s41573-023-00704-7.

Kim, Jin-Ju, Sydney S. Wilbon, and Alessia Fornoni. "Podocyte Lipotoxicity in CKD." *Kidney360* 2, no. 4 (February 26, 2021): 755–62. https://doi.org/10.34067/KID.0006152020.

Koehler, Sybille, Felicitas E. Hengel, Bernhard Dumoulin, Laurel Damashek, Lawrence B. Holzman, Katalin Susztak, and Tobias B. Huber. "The 14th International Podocyte Conference 2023: From Podocyte Biology to Glomerular Medicine." *Kidney International* 105, no. 5 (May 2024): 935–52. https://doi.org/10.1016/j.kint.2024.01.042.

Kohn, Donald B., Yvonne Y. Chen, and Melissa J. Spencer. "Successes and Challenges in Clinical Gene Therapy." *Gene Therapy* 30, no. 10 (November 2023): 738–46. https://doi.org/10.1038/s41434-023-00390-5.

Kopp, Jeffrey B., Hans-Joachim Anders, Katalin Susztak, Manuel A. Podestà, Giuseppe Remuzzi, Friedhelm Hildebrandt, and Paola Romagnani. "Podocytopathies." *Nature Reviews. Disease Primers* 6, no. 1 (August 13, 2020): 68. https://doi.org/10.1038/s41572-020-0196-7.

Li, Bei, Chen Qi, Yifan Zhang, Linru Shi, Jiahui Zhang, Hui Qian, and Cheng Ji. "Frontier Role of Extracellular Vesicles in Kidney Disease." *Journal of Nanobiotechnology* 22, no. 1 (September 20, 2024): 583. https://doi.org/10.1186/s12951-024-02852-3.

Li, Huahua, Jin Zheng, Yacen Wu, Hong Zhou, Suli Zeng, and Quanqing Li. "Dendrobium Officinale Polysaccharide Decreases Podocyte Injury in Diabetic Nephropathy by Regulating IRS-1/AKT Signal and Promoting Mitophagy." *Aging* 15, no. 19 (October 15, 2023): 10291–306. https://doi.org/10.18632/aging.205075.

Li, Ping, Yuqian Xi, Yanping Zhang, Abdus Samad, Wenli Lan, Ya Wu, Jiayu Zhao, Guangxin Chen, Changxin Wu, and Qiuhong Xiong. "GLA Mutations Suppress Autophagy and Stimulate Lysosome Generation in Fabry Disease." *Cells* 13, no. 5 (March 1, 2024): 437. https://doi.org/10.3390/cells13050437.

Li, Xiandeng, Ying Zhang, Xiaodong Xing, Mi Li, Yan Liu, Ajing Xu, and Jian Zhang. "Podocyte Injury of Diabetic Nephropathy: Novel Mechanism Discovery and Therapeutic Prospects." *Biomedicine & Pharmacotherapy* 168 (December 1, 2023): 115670. https://doi.org/10.1016/j.biopha.2023.115670.

Li, Xuezhu, Dipankar Bhattacharya, Yue Yuan, Chengguo Wei, Fang Zhong, Feng Ding, Vivette D. D'Agati, Kyung Lee, Scott L. Friedman, and John Cijiang He. "Chronic Kidney Disease in a Murine Model of Non-Alcoholic Steatohepatitis (NASH)." *Kidney International* 105, no. 3 (March 2024): 540–61. https://doi.org/10.1016/j.kint.2023.12.009.

Li, Yuan, Tong Wu, Hongye Li, Mingming Liu, and Haiyan Xu. "Tanshinone IIA Promoted Autophagy and Inhibited Inflammation to Alleviate Podocyte Injury in Diabetic Nephropathy." *Diabetes, Metabolic Syndrome and Obesity* Volume 17 (July 2024): 2709–24. https://doi.org/10.2147/DMSO.S464015.

Li, Yuting, Jiaojiao Fan, Wenping Zhu, Yujia Niu, Wu, and Aihua Zhang. "Therapeutic Potential Targeting Podocyte Mitochondrial Dysfunction in Focal Segmental Glomerulosclerosis." *Kidney Diseases* 9, no. 4 (March 28, 2023): 254–64. https://doi.org/10.1159/000530344.

Lieshout, Laura P. van, Miranda Rubin, Katrina Costa-Grant, Stacy Ota, Diane Golebiowski, Troy Panico, Eli Wiberg, et al. "A Novel Dual-Plasmid Platform Provides Scalable Transfection Yielding Improved Productivity and Packaging across Multiple AAV Serotypes and Genomes."

Molecular Therapy Methods & Clinical Development 29 (June 8, 2023): 426–36. https://doi.org/10.1016/j.omtm.2023.05.004.

Liu, Gary W., Jeffrey W. Pippin, Diana G. Eng, Shixian Lv, Stuart J. Shankland, and Suzie H. Pun. "Nanoparticles Exhibit Greater Accumulation in Kidney Glomeruli during Experimental Glomerular Kidney Disease." *Physiological Reports* 8, no. 15 (August 2020). https://doi.org/10.14814/phy2.14545.

Liu, Yuting, Ziqi Lin, Yuting Wang, Liuhui Chen, Yuequan Wang, and Cong Luo. "Nanotechnology in Inflammation: Cutting-Edge Advances in Diagnostics, Therapeutics and Theranostics." *Theranostics* 14, no. 6 (April 8, 2024): 2490–2525. https://doi.org/10.7150/thno.91394.

Ma, Kang, Lurui Fang, and Wangwei Kong. "Review of Distribution Network Phase Unbalance: Scale, Causes, Consequences, Solutions, and Future Research Direction," February 10, 2020. https://doi.org/10.36227/techrxiv.11401056.v2.

Ma, Yuhua, Yuwen Hu, Yilin Ruan, Xiaocheng Jiang, Min Zhao, Yuxin Wang, Yanrong Ke, Manman Shi, and Guoyuan Lu. "Astragaloside IV Relieves Passive Heymann Nephritis and Podocyte Injury by Suppressing the TRAF6/NF-Kb Axis." *Renal Failure* 46, no. 2 (December 31, 2024): 2371992. https://doi.org/10.1080/0886022X.2024.2371992.

Makhammajanov, Zhalaliddin, Abduzhappar Gaipov, Askhat Myngbay, Rostislav Bukasov, Mohamad Aljofan, and Mehmet Kanbay. "Tubular Toxicity of Proteinuria and the Progression of Chronic Kidney Disease." *Nephrology Dialysis Transplantation* 39, no. 4 (April 1, 2024): 589–99. https://doi.org/10.1093/ndt/gfad215.

Mann, Nina, and Amar Majmundar. "Meet Two Rising Stars in Kidney Genetics - Boston Children's Answers," n.d.

Maturana, Carola J., Jessica L. Verpeut, Mahdi Kooshkbaghi, and Esteban A. Engel. "Novel Tool to Quantify with Single-Cell Resolution the Number of Incoming AAV Genomes Co-Expressed in the Mouse Nervous System." *Gene Therapy* 30, no. 5 (May 2023): 463–68. https://doi.org/10.1038/s41434-021-00272-8.

Medeiros, Thalia, Robert L. Myette, Jorge Reis Almeida, Andrea Alice Silva, and Dylan Burger. "Extracellular Vesicles: Cell-Derived Biomarkers of Glomerular and Tubular Injury." *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology* 54, no. 1 (January 29, 2020): 88–109. https://doi.org/10.33594/000000207.

Medina Rangel, Paulina X., Elizabeth Cross, Chang Liu, Christopher E. Pedigo, Xuefei Tian, Elena Gutiérrez-Calabrés, Soichiro Nagata, et al. “Cell Cycle and Senescence Regulation by Podocyte Histone Deacetylase 1 and 2.” *Journal of the American Society of Nephrology: JASN* 34, no. 3 (March 1, 2023): 433–50. https://doi.org/10.1681/ASN.2022050598.

Miwa, Takashi, Sayaka Sato, Madhu Golla, and Wen-Chao Song. “Expansion of Anticomplement Therapy Indications from Rare Genetic Disorders to Common Kidney Diseases.” *Annual Review of Medicine* 75 (January 29, 2024): 189–204. https://doi.org/10.1146/annurev-med-042921-102405.

MM Yahya, Shaymaa, and Ghada H. Elsayed. “The Role of MiRNA-34 Family in Different Signaling Pathways and Its Therapeutic Options.” *Gene* 931 (December 30, 2024): 148829. https://doi.org/10.1016/j.gene.2024.148829.

Monte Neto, José Tiburcio do, and Gianna Mastroianni Kirsztajn. “The Role of Podocyte Injury in the Pathogenesis of Fabry Disease Nephropathy.” *Jornal Brasileiro De Nefrologia* 46, no. 3 (2024): e20240035. https://doi.org/10.1590/2175-8239-JBN-2024-0035en.

Montenegro, Francesca, Francesca Giannuzzi, Angela Picerno, Antonella Cicirelli, Emma Diletta Stea, Vincenzo Di Leo, and Fabio Sallustio. “How Stem and Progenitor Cells Can Affect Renal Diseases.” *Cells* 13, no. 17 (January 2024): 1460. https://doi.org/10.3390/cells13171460.

Mou, Xingrui, Sophia M. Leeman, Yasmin Roye, Carmen Miller, and Samira Musah. “Fenestrated Endothelial Cells across Organs: Insights into Kidney Function and Disease.” *International Journal of Molecular Sciences* 25, no. 16 (January 2024): 9107. https://doi.org/10.3390/ijms25169107.

Nong, Shiqi, Xiaoyue Han, Yu Xiang, Yuran Qian, Yuhao Wei, Tingyue Zhang, Keyue Tian, Kai Shen, Jing Yang, and Xuelei Ma. “Metabolic Reprogramming in Cancer: Mechanisms and Therapeutics.” *MedComm* 4, no. 2 (2023): e218. https://doi.org/10.1002/mco2.218.

Oniszczuk, Julie, Fannie Le Floch, Ones Mansour, Michael Alimi, Clémence Le Cœur, Vincent Audard, Dil Sahali, Benjamin Carbonnier, André Pawlak, and Sabrina Belbekhouche. “Kidney–Targeted Drug Delivery Systems Based on Tailor-Made Nanocapsules.” *Chemical Engineering Journal* 404 (January 2021): 126475. https://doi.org/10.1016/j.cej.2020.126475.

Patel, Naomi J., David R. W. Jayne, Peter A. Merkel, Pirow Bekker, Yuqing Zhang, P. Jane McDowell, Joslin Johal, et al. “The Glucocorticoid Toxicity Index-Metabolic Domains, an Abridged Version of the Glucocorticoid Toxicity Index: Post-Hoc Analysis of Data from the ADVOCATE Trial.” *The Lancet Rheumatology* 5, no. 7 (July 1, 2023): e413–21. https://doi.org/10.1016/S2665-9913(23)00131-5.

Peek, Jennifer L., and Matthew H. Wilson. “Cell and Gene Therapy for Kidney Disease.” *Nature Reviews Nephrology* 19, no. 7 (July 2023): 451–62. https://doi.org/10.1038/s41581-023-00702-3.

Qiu, Yang, Chuntao Lei, Jieyu Zeng, Yaru Xie, Yiling Cao, Qian Yuan, Hua Su, Zhentao Zhang, and Chun Zhang. “Asparagine Endopeptidase Protects Podocytes in Adriamycin-Induced Nephropathy by Regulating Actin Dynamics through Cleaving Transgelin.” *Molecular Therapy: The Journal of the American Society of Gene Therapy* 31, no. 11 (November 1, 2023): 3337–54. .

Sealfon, Rachel, Laura Mariani, Carmen Avila-Casado, Viji Nair, Rajasree Menon, Julien Funk, Aaron Wong, et al. “Molecular Characterization of Membranous Nephropathy.” *Journal of the American Society of Nephrology : JASN* 33, no. 6 (June 2022): 1208–21. https://doi.org/10.1681/ASN.2021060784.

Sha, Sha, Andrew J. Maloney, Georgios Katsikis, Tam N. T. Nguyen, Caleb Neufeld, Jacqueline Wolfrum, Paul W. Barone, et al. “Cellular Pathways of Recombinant Adeno-Associated Virus Production for Gene Therapy.” *Biotechnology Advances* 49 (2021): 107764. https://doi.org/10.1016/j.biotechadv.2021.107764.

Słuczanowska-Głabowska, Sylwia, Maria Salmanowicz, Marzena Staniszewska, and Andrzej Pawlik. “The Role of Sirtuins in the Pathogenesis of Psoriasis.” *International Journal of Molecular Sciences* 24, no. 13 (January 2023): 10782. https://doi.org/10.3390/ijms241310782.

Snanoudj, Sarah, Céline Derambure, Cheng Zhang, Nguyen Thi Hai Yen, Céline Lesueur, Sophie Coutant, Lénaïg Abily-Donval, et al. “Genome-Wide Expression Analysis in a Fabry Disease Human Podocyte Cell Line.” *Heliyon* 10, no. 14 (July 2024): e34357. https://doi.org/10.1016/j.heliyon.2024.e34357.

Solovev, Ilya, Alena Sergeeva, Anastasya Geraskina, Mikhail Shaposhnikov, Maria Vedunova, Olga Borysova, and Alexey Moskalev. “Aging and Physiological Barriers: Mechanisms of Barrier Integrity Changes and Implications for Age-Related Diseases.” *Molecular Biology Reports* 51, no. 1 (August 19, 2024): 917. https://doi.org/10.1007/s11033-024-09833-7.

Tao, Yu, Robert T Mallet, Keisa W Mathis, and Rong Ma. “Store-Operated Ca $^{2+}$ Channel Signaling: Novel Mechanism for Podocyte Injury in Kidney Disease.” *Experimental Biology and Medicine* 248, no. 5 (March 2023): 425–33. https://doi.org/10.1177/15353702221139187.

Teh, Yoong Mond, Siti Aisyah Mualif, and Soo Kun Lim. “A Comprehensive Insight into Autophagy and Its Potential Signaling Pathways as a Therapeutic Target in Podocyte Injury.” *The International Journal of Biochemistry & Cell Biology* 143 (February 2022): 106153. https://doi.org/10.1016/j.biocel.2021.106153.

Tseng, Che-Hao, Kavya M. Shah, I.-Jen Chiu, and Li-Li Hsiao. “The Role of Autophagy in Type 2 Diabetic Kidney Disease Management.” *Cells* 12, no. 23 (January 2023): 2691. https://doi.org/10.3390/cells12232691.

Wang, Ting, Yanxia Chen, Zhihong Liu, Jing Zhou, Na Li, Yue Shan, and Yinxi He. “Long Noncoding RNA Glis2 Regulates Podocyte Mitochondrial Dysfunction and Apoptosis in Diabetic Nephropathy via Sponging miR‐328‐5p.” *Journal of Cellular and Molecular Medicine* 28, no. 7 (April 2024): e18204. https://doi.org/10.1111/jcmm.18204.

Wang, Yi, Jianfei Gong, Nuo Heng, Yingfan Hu, Rui Wang, Huan Wang, Wei He, et al. “Melatonin Alleviates Palmitic Acid-Induced Mitochondrial Dysfunction by Reducing Oxidative Stress and Enhancing Autophagy in Bovine Endometrial Epithelial Cells.” *Journal of Animal Science and Biotechnology* 15, no. 1 (August 8, 2024): 108. https://doi.org/10.1186/s40104-024-01064-x.

Wang, Yu, Lu Yu, Yajing Li, Siyu Cha, Lijing Shi, Jinlong Wang, Fengfeng Ge, et al. “Supplemented Gegen Qinlian Decoction Formula Attenuates Podocyte Mitochondrial Fission and Renal Fibrosis in Diabetic Kidney Disease by Inhibiting TNF-α-Mediated Necroptosis, Compared with Empagliflozin.” *Journal of Ethnopharmacology* 334 (November 2024): 118572. https://doi.org/10.1016/j.jep.2024.118572.

Wen, Zihao, Chen Liu, Zihao Teng, Quanyi Jin, Zhihuan Liao, Xuan Zhu, and Shuaidong Huo. “Ultrasound Meets the Cell Membrane: For Enhanced Endocytosis and Drug Delivery.” *Nanoscale* 15, no. 33 (2023): 13532–45. https://doi.org/10.1039/D3NR02562D.

Wharram, Bryan L., Meera Goyal, Jocelyn E. Wiggins, Silja K. Sanden, Sabiha Hussain, Wanda E. Filipiak, Thomas L. Saunders, et al. “Podocyte Depletion Causes Glomerulosclerosis: Diphtheria Toxin-Induced Podocyte Depletion in Rats Expressing Human Diphtheria Toxin Receptor Transgene.” *Journal of the American Society of Nephrology: JASN* 16, no. 10 (October 2005): 2941–52. https://doi.org/10.1681/ASN.2005010055.

Wu, Bangqing, Jingwen Wang, Yi Chen, and Yao Fu. “Inflammation-Targeted Drug Delivery Strategies via Albumin-Based Systems.” *ACS Biomaterials Science & Engineering* 10, no. 2 (February 12, 2024): 743–61. https://doi.org/10.1021/acsbiomaterials.3c01744.

Wu, Qingsi, Jiading Wang, Yuanfang Wang, Ling Xiang, Yulu Tan, Jiaxing Feng, Zhirong Zhang, and Ling Zhang. “Targeted Delivery of Celastrol to Glomerular Endothelium and Podocytes for Chronic Kidney Disease Treatment.” *Nano Research* 15, no. 4 (April 1, 2022): 3556–68. https://doi.org/10.1007/s12274-021-3894-x.

Yang, Ya-Wen, Bibek Poudel, Julia Frederick, Poonam Dhillon, Rojesh Shrestha, Ziyuan Ma, Junnan Wu, et al. “Antisense Oligonucleotides Ameliorate Kidney Dysfunction in Podocyte-Specific APOL1 Risk Variant Mice.” *Molecular Therapy* 30, no. 7 (July 2022): 2491–2504. https://doi.org/10.1016/j.ymthe.2022.04.007.

Ye, Siyang, Meng Zhang, Sydney C. W. Tang, Bin Li, and Wei Chen. “PGC1-α in Diabetic Kidney Disease: Unraveling Renoprotection and Molecular Mechanisms.” *Molecular Biology Reports* 51, no. 1 (February 15, 2024): 304. https://doi.org/10.1007/s11033-024-09232-y.

Zhang, Chao, Michael Rehman, Xin Tian, Steven Lim Cho Pei, Jianlei Gu, Thomas A. Bell, Ke Dong, et al. “Glis2 Is an Early Effector of Polycystin Signaling and a Target for Therapy in Polycystic Kidney Disease.” *Nature Communications* 15 (May 1, 2024): 3698. https://doi.org/10.1038/s41467-024-48025-6.

Zhang, Yi, and Zhi-Ying Wu. “Gene Therapy for Monogenic Disorders: Challenges, Strategies, and Perspectives.” *Journal of Genetics and Genomics*, Human Genetic Disorders, 51, no. 2 (February 1, 2024): 133–43. https://doi.org/10.1016/j.jgg.2023.08.001.

Zhao, Yafei, Qimin Zheng, and Jingyuan Xie. “Exploration of Gene Therapy for Alport Syndrome.” *Biomedicines* 12, no. 6 (June 2024): 1159. https://doi.org/10.3390/biomedicines12061159.

Zheng, Xiaoyi, Lauren Higdon, Alexandre Gaudet, Manav Shah, Angela Balistieri, Catherine Li, Patricia Nadai, et al. “Endothelial Cell-Specific Molecule-1 Inhibits Albuminuria in Diabetic Mice.” *Kidney360* 3, no. 12 (December 29, 2022): 2059–76. https://doi.org/10.34067/KID.0001712022.

Zhong, Suye, Na Wang, and Chun Zhang. “Podocyte Death in Diabetic Kidney Disease: Potential Molecular Mechanisms and Therapeutic Targets.” *International Journal of Molecular Sciences* 25, no. 16 (January 2024): 9035. https://doi.org/10.3390/ijms25169035.