Induced Pluripotent Stem Cells from Polycystic Kidney Disease Patients: A Novel Tool to Model the Pathogenesis of Cystic Kidney Disease

Alexis Hofherr**†† and Michael Köttgen*

*Renal Division, Department of Medicine, University Medical Centre Freiburg, Freiburg, Germany; and †Spemann Graduate School of Biology and Medicine (SGBM) and ‡Faculty of Biology, Albert Ludwigs University, Freiburg, Germany


Polycystic kidney disease (PKD) is the most common inherited kidney disorder in humans, affecting 1 in 400–1000 live births. PKD can be inherited as an autosomal dominant (ADPKD) or autosomal recessive trait (ARPKD). ARPKD is characterized by a rapid progressive course of disease with childhood kidney failure. The defective three-dimensional tissue organization in ADPKD also begins in utero but progresses slowly, and signs of the disease may not be detected until late adulthood. The severity of the renal disease in ADPKD is highly variable, ranging from rare in utero cases with massively enlarged cystic kidneys to cases with adequate kidney function into old age. Although the two disorders present with different dynamics, their pathology eventually converges on ESRD.1

ADPKD is caused by mutations in Polycystic Kidney Disease (PKD1) or PKD2, whereas ARPKD arises from mutations in Polycystic Kidney and Hepatic Disease 1 (PKHD1; #173,900, #613,095, and #263,200 in Online Mendelian Inheritance of Man, http://www.ncbi.nlm.nih.gov/omim/). PKD1 encodes for polycystin-1 (PC1), PKD2 encodes for polycystin-2 (PC2; also known as transient receptor potential PC2, TRPP2), and PKHD1 encodes for fibrocystin/polyductin (FPC). A high level of allelic heterogeneity is found for all PKD genes, with hundreds of unique mutations reported for ADPKD and ARPKD (PKD1, 1923 reported mutations; PKD2, 241 reported mutations; PKHD1, 713 reported mutations; The ADPKD Mutation Database [http://pkdb.mayo.edu] and The ARPKD/PKHD1 Mutation Database [http://www.humgen.rwth-aachen.de/index.php]). The vast majority of mutations is unique to single families, with alleles ranging from predicted loss of function to hypomorphic variants. To date, there is rather limited information on genotype-phenotype correlation. It has been particularly difficult to discriminate between pathogenic nucleotide substitutions and harmless sequence variants based on in silico predictions.

Multiple approaches have been used to define the functional connection between PKD disease genes and PKD disease phenotypes. The most significant progress, including the identification of causative genes and the discovery of the importance of cilia, has been achieved through genetic approaches.2 The major remaining challenge centers on understanding the in vivo functions of the polycystins at the cellular and molecular levels. This undertaking is complicated by two factors. (1) The immediate signaling effectors downstream of polycystins have not yet been identified. (2) PKD is a disease of three-dimensional organ structure, and it is not clear whether in vitro cell-based models can recapitulate polycystin function in vivo. In vitro assays to quantify the function of PKD gene products in a robust fashion are urgently needed to investigate the molecular pathogenesis of PKD and determine the functional impact of distinct patient mutations.

In the current issue of JASN, Freedman et al.3 report the generation and characterization of induced pluripotent stem cells (iPSCs) from patients with ADPKD and ARPKD. To model PKD in human cells, Freedman et al.3 established iPSCs...
from fibroblasts of three patients clinically diagnosed with ADPKD and two newborns with ARPKD.

The controlled reprogramming of somatic cells into pluripotent stem cells has been one of the great turning points in the life sciences. The possibility to derive any given cell phenotype from adult cells, regardless of their origin, has fired the imagination of laymen and scientists alike. Two pioneers in this field, John B. Gurdon and Shinya Yamanaka, were awarded the Nobel Prize in Physiology or Medicine in 2012 for the discovery that mature cells can be reprogrammed to become pluripotent. Cell reprogramming by the introduction of a combination of transcription factors represents an attractive strategy for the generation of patient-derived in vitro cell culture models, which may facilitate the patient-specific molecular analysis of PKD pathogenesis. The artificial generation of renal, hepatic, or vascular cells may help to increase our knowledge of the cellular mechanisms of organ-specific PKD phenotypes. Using iPSCs from PKD patients, several important questions can be addressed. (1) What is the cell biologic effect of a given PKD mutation? (2) May mutations be grouped into classes according to their effect on PKD proteins? (3) Are there any causal therapeutic approaches to be discovered in the broad PKD mutation spectrum?

In the study by Freedman et al., genetic sequencing revealed heterozygous PKD1 mutations (C39Y, R2051P, and E1929X) in the three ADPKD patients and two homozygous PKHD1 mutations (T36M and W2736G) in one ARPKD case. Interestingly, Freedman et al. observed loss of heterozygosity in one of the ADPKD iPSC lines, but the cellular phenotype was not different from haploinsufficient iPSCs. The second newborn fit the clinical profile of ARPKD, with enlarged cystic kidneys, hepatic fibrosis, and pulmonary insufficiency, but had no obvious PKHD1 coding sequence alteration. Dermal fibroblasts of these five patients were reprogrammed into distinct PKD iPSC lines by retroviral transduction of the transcription factors OCT4, SOX2, KLF4, and c-MYC. Pluripotency was validated by directed differentiation into lineages expressing markers of the endoderm (SOX17), mesoderm (FOXF1), and ectoderm (NESTIN). Freedman et al. used this iPSC culture system to evaluate the functional relationship between PKD proteins. Freedman et al. showed that PC1, PC2, and FPC are expressed in undifferentiated pluripotent stem cells. PKD protein levels were not different comparing wild-type with mutant cell lines. However, there was a significant reduction of PC2 at the primary cilia in ADPKD-derived cells, although most of these cell lines still expressed one wild-type copy of PC1. To analyze this phenomenon in a cellular system with greater relevance to human disease, Freedman et al. took advantage of the unique differentiation potential of pluripotent stem cells. Because there is currently no verified protocol for directed differentiation into kidney tubular epithelial cells, the iPSCs were differentiated into somatic epithelial cells and hepatoblasts. Quantitative analysis of PC2 localization in cilia of hepatoblasts corroborated a sizable reduction in ADPKD but not ARPKD mutant cells. Taken together, Freedman et al. propose a model, where heterozygous mutations in PC1 lead to reduced quantities of PC2 in the primary cilium. This observation adds fuel to two debated topics in the PKD field: (1) the requirement of PC1 for PC2 trafficking to cilia and (2) the question of whether haploinsufficiency alone is sufficient for cyst initiation or whether somatic inactivation of the remaining normal allele is always necessary. There is considerable data supporting a molecular recessive model of cyst development, but phenotypes associated with haploinsufficiency have been reported as well. As the work by Freedman et al. states, the “data neither prove nor preclude that second hit mutations are required to cause cystogenesis in vivo, and do not directly address the role of impaired PC2 ciliary localization in vivo.”

Although the functional relevance of the reduction of PC2 in cilia of PKD1-haploinsufficient cells remains to be determined, the study by Freedman et al. is an important first step to model PKD using iPSCs. This raises the question about the next steps to be taken to fully exploit the potential of iPSCs in PKD research, and what can be learned from other diseases and organ systems. There have been a number of excellent examples for in vitro disease modeling using patient-derived iPSCs. In one proof-of-principle study for using iPSCs to capture the physiologic mechanisms of genetic variation, Moretti et al. differentiated iPSCs into cardiomyocytes from individuals with long QT syndrome and observed prolonged action potentials in the ventricular and atrial cells. Using this model system, Moretti et al. uncovered a dominant-negative trafficking defect associated with a particular mutation and identified compounds that attenuated the long QT phenotype. Similar studies have been conducted for type 2 long QT syndrome and rare neurologic diseases. Importantly, these studies also established that the iPSC models can be used to identify complex cytotoxic effects of drugs as well as novel therapeutic approaches. Such success stories highlight the next milestones for the use of iPSCs in PKD research: (1) the identification of factors required for directed differentiation of iPSCs into renal tubular epithelial cells and (2) the establishment of robust quantitative assays to correlate the impact of specific mutations on protein function. Because PKD is a disease of three-dimensional organ structure, multicellular assays, such as three-dimensional cultures of iPSC-derived epithelial cells, might be of particular interest.

In summary, Freedman et al. and Thatava et al. have laid the groundwork for using iPSCs in PKD research. This important step forward will provide novel opportunities to model PKD pathogenesis with human cells with defined patient mutations.

**DISCLOSURES**

None.
Both infectious and noninfectious complications related to vascular access are common and are associated with increased morbidity, mortality, costs, and a reduced patient quality of life.1–3 Complications such as thrombosis and infections account for nearly 30% of hospital admissions in hemodialysis patients and consume a significant proportion of outpatient resources, including vascular access monitoring and diagnostic radiology.4 The substantial burden of vascular access on health and health care costs demands a critical review and intensified prevention efforts to minimize the frequency of these serious health care associated complications.

In this issue of JASN, Ravani et al.5 undertook an important analysis, using data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) to study the temporal risk of infectious (access infections or sepsis from any cause) and noninfectious (dysfunction leading to access interventions) complications over the life of the vascular access. Among incident patients, for all types of accesses, the hazard rate for complications was 5–10 times greater in the first 3–6 months than in later follow-up after access creation. The hazard rate for observing a complication event declined over time, with the greatest decline observed among patients using a fistula compared with those using a graft or catheter.

There are other important results to highlight. Surprisingly, the majority of patients (65%) started dialysis with a temporary catheter. Additionally, with a median follow-up of 14 months, 37% and 15% of surviving patients required a second and third access creation, respectively. The rate of noninfectious complications was 10 times higher than that of infectious complications and was primarily related to thrombosis (10,452 noninfectious events and 1131 infectious events in 112,085 patient-months). Complication rates per 1000 access-days, in the first month, were highest for catheters (22 noninfectious events and 2.7 infections), than in grafts (13.4 non- infectious events and 1.8 infections) and fistulas (0.32 noninfectious events and 0.03 infectious). After 3 months, the infectious rates were significantly lower and tended to be less than 0.6, 0.3, and 0.2 per 1000 access-days for catheters, grafts, and fistulas, respectively. Notably, noninfectious complications recurred in almost 50% of the patients using the same access, with a similar pattern being observed for those with infectious complications. Finally, second and subsequent accesses had a substantial increase in the risk of complications compared with the initial access: 35%–58% for noninfectious risk and 51%–85% for infectious risk.

The outcomes of this study are difficult to compare with the existing literature because of the use of nonstandardized definitions. Ravani and colleagues reported infectious complications were evenly divided between access infection and all-cause sepsis. In the U.S. Renal Data System (USRDS) data, the rate of sepsis is higher than the infection rate for all access types; the catheter sepsis rate is 1.6 times greater than catheter infectious rates.6 Similarly, incident patients in the USRDS using a catheter were 3.8 times more likely to have a catheter-related infection than to have a graft or fistula.7