Research Paper

Fatty Acid Oxidation is Impaired in An Orthologous Mouse Model of Autosomal Dominant Polycystic Kidney Disease

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A B S T R A C T

Background: The major gene mutated in autosomal dominant polycystic kidney disease was first identified over 20 years ago, yet its function remains poorly understood. We have used a systems-based approach to examine the effects of acquired loss of *Pkd1* in adult mouse kidney as it transitions from normal to cystic state.
Methods: We performed transcriptional profiling of a large set of male and female kidneys, along with metabolomics and lipidomics analyses of a subset of male kidneys. We also assessed the effects of a modest diet change on cyst progression in young cystic mice. Fatty acid oxidation and glycolytic rates were measured in five control and mutant pairs of epithelial cells.
Results: We find that females have a significantly less severe kidney phenotype and correlate this protection with differences in lipid metabolism. We show that sex is a major determinant of the transcriptional profile of mouse kidneys and that some of this difference is due to genes involved in lipid metabolism. *Pkd1* mutant mice have transcriptional profiles consistent with changes in lipid metabolism and distinct metabolite and complex lipid profiles in kidneys. We also show that cells lacking *Pkd1* have an intrinsic fatty acid oxidation defect and that manipulation of lipid content of mouse chow modifies cystic disease.
Interpretation: Our results suggest PKD could be a disease of altered cellular metabolism.

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1. Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD; MIM ID's 173900, 601313, 613095) is estimated to affect almost 1/1000 and is the most common genetic cause of end stage renal disease (Torres et al., 2007). While advances have been made in slowing the progression of some other forms of chronic kidney disease, standard treatments have not reduced the need for renal replacement therapy in ADPKD (Spithoven et al., 2014). Unfortunately, several experimental interventions also have recently failed to show significant benefit in slowing the rate of functional decline (Serra et al., 2010; Walz et al., 2010; Schrier et al., 2014; Torres et al., 2014), and the only positive study reported very modest effects (Torres et al., 2012). These findings suggest new treatment strategies are required.

A central dogma of molecular genetics is that discovery of the causative genes will lead to identification of key pathways and potential targets for intervention. In the case of ADPKD, the two genes mutated in the disorder, *PKD1* and *PKD2*, were identified almost 20 years ago and yet their functions remain poorly understood. The *PKD1* gene product, polycystin-1 (PC1), encodes a large membrane protein that requires the *PKD2* gene product, polycystin-2 (PC2), for its trafficking to the

primary cilium where the two are thought to form a receptor channel complex (Kim et al., 2014; Cai et al., 2014). What the complex senses and what it signals remains controversial. The primary cilium has emerged as a key player in the pathogenesis of PKD as mutations in dozens of different genes that encode either essential ciliary components or factors in ciliary signaling pathways result in PKD. A recent report suggests that the relationship between the polycystin complex and ciliary signaling is complicated, however. While ablation of primary cilia by mutation of core ciliary components results in cysts, these same perturbations done in the setting of *Pkd1* or *Pkd2* inactivation results in significant attenuation of cystic disease (Ma et al., 2013). These data suggest that the polycystin complex provides a suppressive signal for a novel, cilia-dependent growth-promoting pathway that is independent of MAPK/ERK, mTOR, or cAMP pathways, three effector pathways previously implicated as major drivers of cyst growth. The identities of the growth-promoting and growth-inhibiting pathways remain unknown.

We have taken a systems-based approach to study *Pkd1* gene function. Building on our previous work identifying markedly different outcomes in animals with induced *Pkd1* inactivation before or after P12 and correlating this susceptibility with metabolic status (Piontek et al., 2007; Menezes et al., 2012), we now show that female sex is partially protective in adult-induced *Pkd1* inactivation, that sex differences in metabolic status may account for this effect, and that cells

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lacking *Pkd1* have abnormal fatty acid oxidation. Finally, manipulating diet in *Pkd1* mouse models, we demonstrate a positive correlation between lipid content in mouse chow and cystic kidney disease severity. Our results therefore suggest that abnormal lipid metabolism is an intrinsic component of PKD and an important modifier of disease progression.

2. Materials and Methods

2.1. Ethics Statement

All studies were performed using protocols approved by NIH Animal Care and Use Committee, and mice were kept and cared in pathogen-free animal facilities accredited by the American Association for the Accreditation of Laboratory Animal Care and meet federal (NIH) guidelines for the humane and appropriate care of laboratory animal.

2.2. Animal Studies

Fifth-generation C57/BL6 *Pkd1*[tm2Ggg] (Piontek et al., 2004) mice were crossed to the reporter mice C57/BL6 congenic B6.129S4-Gt(ROSA)26Sortm1Sor/J (stock 003474, Jackson Laboratories) and to C57/BL6 tamoxifen-Cre (B6.Cg-Tg(Cre/Esr1)5Amc/J mice (stock 004682), Jackson Laboratories) or Ksp-Cre B6.Cg-Tg(Cdh16-cre)91Igr/J(stock 012237), Jackson Laboratories). For early-onset diet studies in tamoxifen-Cre mice, nursing moms of P7 mice were induced by one intraperitoneal injection of 0.2 mg/g tamoxifen (Sigma, T5648) in corn oil (Sigma-Aldrich, C8267) and pups were harvested at P21. In the Ksp-Cre line, mice were harvested at P14. In both studies, breeding pairs and offspring were continually fed with either NIH31 or NIH37 diets for the duration of the study. For the late-onset studies, adult mice were induced at P40 by one intraperitoneal injection of 0.2 mg/g tamoxifen in corn oil and harvested between P86 and P210. Induced, tamoxifen-cre negative, *Pkd1*[tm2Ggg] (referred to as *Pkd1*[cko/cko] in this report) mice were considered controls.

2.3. Cell Lines

Kidneys from two *Pkd1*[cko/cko] animals (121112-C: male, <P12; and 94414: male, P463) were harvested, minced and digested using a collagenase/hyaluronidase solution (Stemcell technologies, cat. no. 07912) followed by proximal or collecting/distal tubule cell enrichment using, respectively, biotinylated *Lotus tetragonolobus* Lectin (LTL) (Vector Laboratories, cat. no. B-1325) or biotinylated *Dolichos biflorus* Agglutinin (DBA) (Vector Laboratories, ca. no. B-1035) and Cellection Biotin Binder kit (ThermoFischer, cat. no. 11533D). Cells were immortalized using the large T antigen (Addgene plasmid no. 22298). *Pkd1* was conditionally inactivated using cre recombinase (121112C-LTL cells; Excellgen, cat. no. EG-1001) or viral transduction (121112-C DBA and 94414-LTL/DBA cells) using LV-Cre (Addgene plasmid no. 12106). At the time of inactivation, a corresponding control was generated using viral transduction with plasmid LV-Lac (Addgene plasmid no. 12108). *Pkd1* inactivation was confirmed using genomic PCR and/or reverse-transcriptase PCR (TaqMan gene expression assay, Applied Biosystems, cat. no. 4351372, Mm00465436_g1). mCCDcl1 (mCCD) cells were a kind gift from the Rossier lab (Gaeggeler et al., 2005). mCCD *Pkd1* knock-down cells were generated using viral transduction with the shRNA clone TRCN0000072085 and the corresponding pLKO.1 TRC21 control (Addgene plasmid 10,879). Cells were grown in DMEM/F12 media (Life cat. no. 21041–025) with 2% FBS (GEMINI Bio-Products cat. no. 100–106), 1X Insulin-Transferrin-Selenium (Thermo Fisher Scientific, cat. no. 41400–045), 5 uM dexamethasone (SIGMA, cat. no. D1756), 10 ng/ml EGF (SIGMA, cat. no. SRP3196), 1 nM 3,3′,5-Triiodo-L-thyronine (SIGMA, cat. no. T6397) and 10 mM HEPES (CORNING, cat. no. 25-060-CI).

Mouse embryonic fibroblasts (MEFs) were obtained from E12.5 and E13.5 *Pkd1* knockout (*Pkd1*[tm1GGG] (Bhunia et al., 2002)) and control mice. Briefly, whole embryos were minced, washed in PBS and cultured in six-well tissue culture plate in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). A total of 6 MEF lines was used for this study: three from E13.5 mouse littermates (2 *Pkd1*[ko/ko] and 1 *Pkd1*[wt/wt]) immortalized using large T antigen (Addgene plasmid no. 22,298) and an additional set of three primary (passage 2, non immortalized) E12.5 embryos (1 *Pkd1*[ko/ko] and 2 *Pkd1*[wt/wt] littermate controls).

2.4. Phenotypic Characterization

Kidneys and body weight were measured and the mean kidney/body weight ratio was the readout for disease severity. The left kidney was fixed in 4% paraformaldehyde, centrally sectioned along the longitudinal axis and processed for histology. The right kidney was snap frozen in liquid nitrogen.

2.5. mRNA Expression Studies

Eighty mouse kidneys with induced deletion of *Pkd1* at P40 were harvested between 102 and 210 days of age (14 control females, 21 mutant females, 19 control males and 26 mutant males). Total RNA was isolated using Trizol (Life, cat. no. 15596-018) followed by RNeasy plus kit (Qiagen, cat. no. 74136). Further sample processing for microarray analysis was performed by the University of Chicago Genomics Facility, Knapp Center for Biomedical Discovery, following the facility's protocols, and hybridized to Illumina's MouseRef-8 v2.0 BeadChip expression arrays. These data have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE72554 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72554).

For RT-PCR, cDNA was generated using Superscript Vilo (Life, cat. no. 11755050) amplified with the expression assay Mm00465436_g1 (TaqMan gene expression assay, Applied Biosystems, cat. no. 4351372), normalized to Gapdh and analyzed using the delta–delta-Ct method. Each sample was analyzed in 2 independent experiments, each done with 3 or 4 replicates.

2.6. Statistical Analysis

Comparison of kidney/body weight (KBW) mutant male vs. female curves was performed using generalized linear model in R (Team, 2014) and the effect size was estimated calculating Cohen's d with the compute.es package in R. Analysis of KBW in mutants fed NIH31 or NIH37 was done using Student's t test. Real time PCR data was analyzed fitting an anova model including within subject error. Fatty acid oxidation assay results for five technical (well) replicates were averaged for each cell line and genotype. The corresponding five pairs of means were analyzed using paired t-test.

2.7. Gene Array Expression Analyses

Raw Illumina gene expression data were processed using variance stabilization and quantile normalization with the lumi package (Du et al., 2008; Lin et al., 2008) in R (Team, 2014), filtered to include only probes with detection call p value <0.05 in at least seven of the arrays, followed by batch-removal using COMBAT (Johnson et al., 2007). Same-gene probes were consolidated to a single identifier keeping the probe with highest mean value. Detection of differentially expressed genes was performed using linear models and empirical Bayes methods implemented in the limma package (Smyth, 2004) in R. For time course analysis, splines and limma packages in R were used to fit separate curves in mutant males and females for each gene, and then the parameters corresponding to the interaction of time and sex were evaluated to