Research & Discovery Grand Rounds

Polycystic Kidney Disease: Pathogenesis to Therapeutics

James P. Calvet
March 5, 2018
Objectives

To Understand:

1. The genetics and pathogenesis of ADPKD
2. The role of decreased Ca2+ in ADPKD
3. The role of cyclic AMP in ADPKD
4. The therapeutic basis for V2R antagonists
Polycystic Kidney Disease Research

- **TCF**
- **b-catenin**
- **Gα12**
- **NH₂**
- **COOH**
- **polycystin-1**
- **polycystin-2**
- **Ca²⁺**
- **NFAT**
- **β-catenin**
- **TCF**
- **4 3 2**
- **3.3 kb**
- **GTTCCTTTGTTTTT**
- **GL3 - wt TBE1**
- **PKD**
- **POLYCYSTIN LOSS**
- **Ca²⁺ restriction**
- **cAMP**
- **Ca²⁺**
- **NFAT**
- **Normal**
- **PKD**
- **Decreased cell proliferation in vitro and normal tubule formation in vivo**
- **Increased cell proliferation in vitro and cyst formation in vivo**

Graphs and images showing various aspects of polycystic kidney disease research, including experimental data, molecular biology diagrams, and histological sections of kidneys.
Autosomal dominant polycystic kidney disease (ADPKD)

- Incidence 1:500-1,000
- 600,000 cases in U.S.
- 12.5 M cases worldwide

- End stage renal disease
- Extra-renal cysts
- Hypertension
- Vascular aneurysms

- There are no effective FDA approved therapies for PKD
Autosomal dominant polycystic kidney disease (ADPKD)

PKD1

PKD2

Figure adapted from:
Autosomal dominant polycystic kidney disease (ADPKD)

PKD1

(~85%)

PKD2

Figure adapted from:
Autosomal dominant polycystic kidney disease (ADPKD)

PKD1 (~85%)

PKD2 (~15%)

Figure adapted from:
Autosomal dominant polycystic kidney disease (ADPKD)

PKD1

(~85%)

PKD2

(~15%)

Figure adapted from:
Autosomal dominant polycystic kidney disease (ADPKD)

PKD1

PKD2

Figure adapted from:
Heterotrimeric G-proteins

Phosphatase

Kinase

PC1

PC2


Autosomal dominant polycystic kidney disease (ADPKD)

PKD1

PKD2

Figure adapted from:
Signaling Targets in ADPKD

Cilia Disorders – Ciliopathies
Figure 1. Structure of the Cilium and Intraflagellar Transport.

The cilium is a hairlike structure on the cell surface that consists of a microtubule-based axoneme covered by a specialized plasma membrane, which is assembled from the basal body, or mother centriole. Transition fibers act as a filter for molecules passing into or out of the cilium. Nephrocystin-1 is localized at the transition zone of epithelial cells (not shown). Axonemal and membrane components are transported in raft macromolecular particles (complexes A and B) by means of intraflagellar transport (IFT) along the axonemal doublet microtubules toward the tip complex by heterotrimeric kinesin-2. Mutations of KIF3a cause renal cysts and aplasia of the cerebellar vermis in mice. Retrograde transport occurs by means of the motor protein cytoplasmic dynein. (Adapted from Bisgrove and Yost.)
Intraflagellar Transport (IFT) Complexes
Renal Tubule Cilium

Collecting Duct Cilia
Localization of polycystin-1 to primary cilium of M-1 renal epithelial cells

Shirin Sundar
# Ciliopathies

## Table 1: Cystic diseases of the kidney: their causal genes, encoded proteins, localization, and proposed function

<table>
<thead>
<tr>
<th>Disease</th>
<th>Main features</th>
<th>Genes involved</th>
<th>Corresponding protein</th>
<th>Protein localization</th>
<th>Postulated functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal recessive PKD (ARPKD)</td>
<td>Renal cysts, enlarged kidneys, hepatic fibrosis</td>
<td>PKHD1</td>
<td>Fibrocystin/polyductin (FPC)</td>
<td>Cilia and secreted</td>
<td>Calcium response; proliferation/differentiation</td>
</tr>
<tr>
<td>Autosomal dominant PKD (ADPKD)</td>
<td>Renal, hepatic, pancreatic and brain cysts</td>
<td>PKD1, PKD2</td>
<td>Polycystin 1 (PC1) Polycystin 2 (PC2)</td>
<td>Cilia, Golgi apparatus, focal adhesions</td>
<td>Calcium response; proliferation/differentiation</td>
</tr>
<tr>
<td>Nephronophthisis (NPHP) and Senior Loken Syndrome (SNLS)</td>
<td>Renal fibrosis, renal cysts, tubular atrophy, renal dystrophy (in SNLS)</td>
<td>NPHP1–NPHP11</td>
<td>Nephrocystin-1, 2/inversin, 3, 4, 5, 6/CEP290, 7/GLIS2, 8/RPRIP1L, 9/NEK8, 11/Mecckel</td>
<td>Cilia, basal bodies, centrosomes, focal adhesions.</td>
<td>Cell–cell and cell–matrix adhesion; actin cytoskeleton; cell division; Wnt and Shh signaling</td>
</tr>
<tr>
<td>Joubert syndrome (JS)</td>
<td>NPHP and cerebellar ataxia</td>
<td>AH11, NPHP1, CEP290, JBT5/TEM67, RPRIP1L, ARL13B, CC2D2A, INPP5E, JBT5/TEM67</td>
<td>Joubertin, Nephrocystin, CEP290, Meckelin, RPRIP1L, ARL13B, CC2D2A, INPP5E, TEM216</td>
<td>Cilia, basal bodies, centrosomes, cell junctions</td>
<td>Ciliogenesis, Sonic Hedgehog signaling</td>
</tr>
<tr>
<td>Bardet–Biedl syndrome (BBS)</td>
<td>Renal cysts, obesity, polydactyly, renal dystrophy, mental retardation</td>
<td>BBS1-12, MKS1, CEP290, FRITZ, SDCCAG8</td>
<td>BBS1-12, MKS1, CEP290, FRITZ, SDCCAG8</td>
<td>Centrosomes, basal bodies</td>
<td>Pericentriolar organization, ciliogenesis, Wnt signaling</td>
</tr>
<tr>
<td>Meckel–Gruber syndrome (MKS)</td>
<td>Occipital meningocoecephaly, cystic kidneys, liver fibrosis, polydactyly</td>
<td>MKS1, MKS3/TEM67, NPHP3, CEP290, RPRIP1L, CC2D2A, MKS2/TEM216</td>
<td>MKS1, meckelin, nephrocystin 3, CEP290, RPRIP1L, CC2D2A, TEM216</td>
<td>Centrosomes, cilia, plasma membrane</td>
<td>Basal body localization, ciliogenesis, Hedgehog signaling</td>
</tr>
<tr>
<td>Oral–facial–digital syndrome 1 (OFD1)</td>
<td>Malformations of face, oral cavity and digits, renal cysts, polydactyly</td>
<td>OFD1</td>
<td>OFD1</td>
<td>Cilia, basal bodies, centrosomes, nucleus</td>
<td>Ciliogenesis, L-R asymmetry, possibly gene regulation</td>
</tr>
<tr>
<td>Short-Rib Polydactyly (incl. Jeune Asphyxiating Thoracic Dystrophy)</td>
<td>Renal cysts, shortened bones, polydactyly, situs inversus</td>
<td>DYN2CH1, IFT80</td>
<td>Cytoplasmic dynein 2 heavy chain, IFT80</td>
<td>Chondrocyte cilia, basal bodies</td>
<td>Intraflagellar transport, Hedgehog signaling</td>
</tr>
<tr>
<td>Uromodulin-associated kidney diseases (MCKD2, FJHN, GCKD)</td>
<td>Renal cysts, fibrosis, hyperplasmosis, hypopurinemicemia</td>
<td>UMOD, MCKD1, MCKD2</td>
<td>Uromodulin</td>
<td>Cilia, basal bodies, centrosomes, secreted</td>
<td>Unknown ciliary role</td>
</tr>
</tbody>
</table>

PKD, Polycystic kidney disease; MCKD2, medullary cystic kidney disease type 2; FJHN, familial juvenile hyperuricemic nephropathy; GCKD, glomerulocystic kidney disease.
Ciliopathies

Neoplasia Renal Cysts

Developmental Disorders

PKD

Cancer

VHL – cysts

APC – cysts

MYC – cysts

VHL – CCRCC

APC – FAP/CRC

MYC – cancers

BBS

MKKS

SLSN

PCD

MKS

NPHP

JATD

OFD

EVC

RP

JS
Cyst Growth in PKD


Increased cell proliferation is critical for cyst growth in PKD
Cyst Growth in PKD


Increased cell proliferation is critical for cyst growth in PKD

C-MYC overexpression is a hallmark of PKD
Initiation event

Normal tubule

Area of abnormal cell proliferation

Dilating tubule

Continued cell proliferation and fluid secretion

Isolated cyst

Cyst
Exponential kidney growth, ~5% per year

Glomerular filtration rate (mL/min/1.73 m²)

Total kidney volume (mL)

Age (years)

Compensatory hyperfiltration

Rapid decline in GFR, ~10 mL/min/yr

ESRD

Compression of tubular and vascular structures

Inflammation

Fibrosis

Acquired cystic disease
- Disease progression is variable
  - Allelic
  - Background
  - Environment
- Therapy – to slow progression
• Disease progression is variable
  • Allelic
  • Background
  • Environment

Grantham et al., NEJM 2006, 354;20
• Disease progression is variable
  • Allelic
  • Background
  • Environment
• Therapy – to slow progression

Goal of therapy is to “bend the curve” to extend lifetime of kidneys
Initiation event

Area of abnormal cell proliferation

Continued cell proliferation and fluid secretion

Cyst
Cell Proliferation
The Roles of Cyclic AMP and Calcium in PKD


ADPKD kidney

PKD Biomarkers and Biomaterials Core
Darren Wallace, Ph.D., Director
Dr. Wallace Dissecting a Human ADPKD Kidney

PKD Biomarkers and Biomaterials Core
Darren Wallace, Ph.D., Director
Baseline Intracellular Calcium Concentrations in Normal and ADPKD Cells


Lower Ca\(^{2+}\)
Cyclic AMP-dependent cell proliferation in PKD

Cell Number

Cyclic AMP

Anti-Mitogenic

Normal

Time

Normal
Cyclic AMP-dependent cell proliferation in PKD

ADPKD

Normal

Cell Number

Mitogenic

Anti-Mitogenic

Normal

Time

Cyclic AMP

ADPKD
Cyclic AMP-dependent cell proliferation in PKD

Calcium channel blocker (CCB)
Verapamil

Cyclic AMP

Mitogenic
Normal Cells
Behave Like
ADPKD Cells

Normal

Cell Number

Time
PKD Phenotypic Switch

NORMAL POLYCYSTINS

- cAMP
- Growth Factors
- PKA
- PKA
- Ras
- Akt
- PI3K
- PI3K
- MEK
- MEK
- ERK
- ERK
- Decreased cell proliferation

LOSS OF POLYCYSTINS or CALCIUM DECREASE

- cAMP
- Growth Factors
- Ca$^{2+}$
- Ca$^{2+}$
- PKA
- PKA
- Ras
- Akt
- PI3K
- PI3K
- MEK
- MEK
- ERK
- ERK
- Increased cell proliferation

- cAMP is Harmless
- cAMP is Mitogenic
PKD Phenotypic Switch

**NORMAL POLYCYSTINS**

- cAMP
- Growth Factors
- Ca^{2+}
- PKA
- PI3K
- Ras
- Akt
- Raf-1
- B-Raf
- MEK
- ERK
- Decreased cell proliferation

**LOSS OF POLYCYSTINS or CALCIUM DECREASE**

- CALCIUM INCREASE
  - • Bay-K8644
  - • A23187

**LOSS OF POLYCYSTINS**

- cAMP
- Growth Factors
- Ca^{2+}
- PKA
- PI3K
- Ras
- Akt
- Raf-1
- B-Raf
- MEK
- ERK
- Increased cell proliferation
Calcium Reduction Exacerbates Renal Cystic Disease

Initiation event

Area of abnormal cell proliferation

Continued cell proliferation and fluid secretion

Cyst

Normal tubule

Dilating tubule

Isolated cyst
Fluid Secretion
The Role of Cyclic AMP in PKD


Collecting Duct Cysts Predominate in ADPKD
Cyst
Cyst
Cyst
Single Cell in a PKD Cyst

Inside of cyst

Blood side
Fluid Secretion in PKD

Inside of cyst

Blood side

Cl⁻

CFTR

NKCC1

Chloride
Fluid Secretion in PKD

Inside of cyst

Blood side

Chloride

CFTR

Cyclic AMP

NKCC1

Cl⁻
Fluid Secretion in PKD

Chloride

CFTR

Cyclic AMP

NKCC1

Sodium

Sodium

Chloride
Fluid Secretion in PKD

NaCl

NaCl

NaCl

NaCl

Cyclic AMP

CFTR

NKCC1

Cl⁻

Water

Water
Cyclic AMP Drives Cyst Enlargement in Pkd1 -/- Embryonic Kidneys

Cyst enlargement is CFTR- and NKCC1-dependent

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>E15.5</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
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</thead>
<tbody>
<tr>
<td>Pkd1</td>
<td></td>
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<tr>
<td>CFTR</td>
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<tr>
<td>NKCC1</td>
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<td>−/− +/+</td>
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<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
<td>+/+</td>
</tr>
</tbody>
</table>

Brenda Magenheimer
Fluid Secretion in PKD

- NaCl
- NaCl
- NaCl
- NaCl
- Cyclic AMP
- CFTR
- NKCC1
- Water

Red arrows indicate the direction of movement.
Initiation event leads to a normal tubule. Further dilation causes an area of abnormal cell proliferation. Continued cell proliferation and fluid secretion lead to a cyst. Cyclic AMP (cAMP) is involved in these processes.
Fluid Secretion in PKD

NaCl

NaCl

NaCl

NaCl

CFTR

NKCC1

Cyclic AMP

Cl⁻

Vasopressin

VASOPRESSIN RECEPTOR

Water

Water
Adenylate Cyclases

Phosphodiesterases

AMP
Adenosine Monophosphate

Adenylate Cyclases

cAMP
Cyclic Adenosine Monophosphate

Phosphodiesterases
PDE’s

Balance Between Adenylate Cyclases and Phosphodiesterases
Decreased Phosphodiesterase Activity in PKD

Pinto CS, Raman A, Reif GA, Magenheimer BS, White C, Calvet JP, Wallace DP
Phosphodiesterase Isoform Regulation of Cell Proliferation and Fluid Secretion in Autosomal Dominant Polycystic Kidney Disease.

Adenylate Cyclases

[Diagram showing the relationship between AMP, cAMP, and Phosphodiesterases (PDE1 and PDE4)]

PKD mutation

Decreased Phosphodiesterase Activity

Calcium
Adenylate Cyclases

Arginine Vasopressin

Arginine Vasopressin Receptor

AMP
Adenosine Monophosphate

cAMP
Cyclic Adenosine Monophosphate

Phosphodiesterases
PDE1 and PDE4

PKD mutation

Calcium
Adenylate Cyclases

Arginine Vasopressin

Arginine Vasopressin Receptor

Adenylate Cyclases

AMP
Adenosine Monophosphate

cAMP
Cyclic Adenosine Monophosphate

Phosphodiesterases
PDE1 and PDE4

Calcium

PKD mutation

V2R Antagonist
Gattone VH, Maser RL, Tian C, Rosenberg JM, Branden MG.
United States Patent

Gattone, II

Treatment of polycystic kidney disease using vasopressin V.sub.2 receptor antagonists

Abstract

The present invention is directed to the novel treatment of ARPKD and ADPKD by administering a pharmacologically effective amount of a V.sub.2 receptor antagonist. Orally active V.sub.2 receptor antagonists such as OPC-31260, OPC-41061, SR121463A and VPA-985 are administered alone, or in combination to mammalian PKD subjects to reduce the cAMP generated by the increased expression of AVP-V.sub.2 receptor, AQP2 and AQP3, thereby reducing and/or preventing cyst enlargement.

Inventors: Gattone, II; Vincent H. (Overland Park, KS)
Assignee: University of Kansas Medical Center (Kansas City, KS)
Family ID: 26750134
Appl. No.: 09/211,396
Filed: December 14, 1998

Vince Gattone (1951-2014)
1. Abnormal proliferation

2. Cyst-filling fluid secretion

Both pathogenic processes are driven by cyclic AMP

Tolvaptan

Cyclic AMP

Cyclic AMP

1. Abnormal proliferation

Tolvaptan
Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease

Vicente E. Torres, M.D., Ph.D., Arlene B. Chapman, M.D., Olivier Devuyst, M.D., Ph.D., Ron T. Gansevoort, M.D., Ph.D., Jared J. Grantham, M.D., Eiji Higashihara, M.D., Ph.D., Ronald D. Perrone, M.D., Holly B. Krasa, M.S., John Ouyang, Ph.D., and Frank S. Czerwiec, M.D., Ph.D., for the TEMPO 3:4 Trial Investigators*
1. Role of decreased Ca2+ cell proliferation
2. Role of cAMP in cell proliferation and fluid secretion
3. Role of decreased Ca2+ in raising cAMP levels
Disease progression is variable
- Allelic
- Background
- Environment
- Therapy – to slow progression

Goal of therapy is to “bend the curve” to extend lifetime of kidneys
Therapies in pre-clinical and/or clinical trials to lower cyclic AMP

- Tolvaptan
- Octreotide
- Lanreotide (Somatostatin analogs)
- Increased water intake

Other therapies in pre-clinical and/or clinical trials

- mTOR inhibitors
- Niacinamide
- H2-Gamendazole
Inhibition of sirtuin-1 ameliorates PKD cyst growth

Inhibition of sirtuin-1 ameliorates PKD cyst growth

Lonidamine Analogs and Treatment of Polycystic Kidney Disease
Date of Patent: January 29, 2013.

Lonidamine (LND)

H2-Gamendazole (H2-GMZ)
A sample of the chemical compound called H2-gamendazole that was developed by Joseph Tash, a researcher at Kansas University Medical Center. The compound temporarily stops the production of sperm, and could be used as a means of birth control.


Gamendazole, an orally active indazole carboxylic acid male contraceptive agent, targets HSP90AB1 (HSP90BETA) and EEF1A1 (eEF1A), and stimulates Il1a transcription in rat Sertoli cells.


Hsp90 was found to be a direct target of Gamendazole

Photo by Kevin Anderson. Kevin Anderson Photography. A sample of the chemical compound called H2-gamendazole that was developed by Joseph Tash, a researcher at Kansas University Medical Center. The compound temporarily stops the production of sperm, and could be used as a means of birth control.
Parallel Signaling Pathways Implicated in PKD

Pathway Intermediates

- ErbB2 EGFR
- cAMP
- Chloride
- IGF-1R

Effects on Cyst Growth

- Cell Proliferation
- Fluid Secretion
- Cell Growth

Signaling pathways in PKD. Shown are the major signaling pathways that have been implicated in PKD, that lead to abnormal activation of cell proliferation, fluid secretion, and increased cell growth. Hsp90 client proteins are circled. All of these pathways are currently being tested with single-target inhibitors such as inhibitors of EGFR, Src, Raf, MEK, Cdk4, CFTR, and mTOR. It is proposed that Hsp90 inhibition will target multiple, parallel pathways, at multiple effectors within each pathway, thus more effectively slowing cell growth and proliferation, and cyst-filling fluid secretion.
H2-Gamendazole inhibits cAMP-mediated fluid secretion

**Pkd1 Genotype**

- **+/−**
  - E 15.5
  - Day 1
  - Day 2
  - Day 3
  - Day 4

- **+/-**
  - +5 μM H2-GMZ

- **−/−**
  - +5 μM H2-GMZ

**Brenda Magenheimer**

100 μM 8-Br-cAMP
H2-GMZ inhibits cyst growth in a Pkd1\textsuperscript{flox/flox}; Pkhd1-Cre mouse model

Daily i.p. injections
20 mg/kg H2-GMZ
Postnatal day 8-18

Brenda Magenheimer
Shirin V. Sundar
Gail Reif
Darren P. Wallace
Joseph S. Tash
Gunda I. Georg
Julie Zhou
Xiaogang Li
James P. Calvet
Treatment with H2-GMZ increases the survival time of $Pkd1^{flox/flox} \cdot Pkd1^{Cre}$ mice.

Average survival time
Control vs H2-GMZ: $28.8 \pm 5$ vs $67.8 \pm 23$
$p < 0.01$
Disease progression is variable
- Allelic
- Background
- Environment

Therapy – to slow progression

Goal of therapy is to “bend the curve” to extend lifetime of kidneys
Thank You!
Thank You

PKD Foundation
KU Cancer Center
KUMC Research Institute
National Institutes of Health
Jared Grantham Kidney Institute
Institute for Advancing Medical Innovation
QUESTIONS?