

DATASET BRIEF

Protein composition of liver cyst fluid from the BALB/c-cpk/+ mouse model of autosomal recessive polycystic kidney disease

Xianyin Lai¹, Bonnie L. Blazer-Yost^{1,2,3}, Vincent H. Gattone II², Monalisa N. Muchatuta³ and Frank A. Witzmann¹

¹Department of Cellular & Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN, USA

²Department of Anatomy & Cell Biology, Indiana University School of Medicine, Indianapolis, IN, USA

³Department of Biology, Indiana University Purdue University at Indianapolis, Indianapolis, IN, USA

Cysts arising from hepatic bile ducts are a common extra-renal pathology associated with polycystic kidney disease in humans. As an initial step in identifying active components that could contribute to disease progression, we have investigated the protein composition of hepatic cyst fluid in an orthologous animal model of autosomal recessive polycystic kidney disease, heterozygous (BALB/c-cpk/+) mice. Proteomic analysis of cyst fluid tryptic digests using LC-MS/MS identified 303 proteins, many of which are consistent with enhanced inflammatory cell processes, cellular proliferation, and basal laminar fibrosis associated with the development of hepatic bile duct cysts. Protein identifications have been submitted to the PRIDE database (<http://www.ebi.ac.uk/pride>), accession number 9227.

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Polycystic kidney disease (PKD) is a heritable disease characterized by the development of cysts in the kidney and liver. PKD is the third leading cause of kidney failure in the United States [1] and exists in two forms, autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). ADPKD is the most common form and is usually diagnosed after the fourth decade of life. Typically diagnosed in neonates and children, ARPKD is a less common but more severe form of the disease. In the progression of ARPKD, liver cysts develop from cholangiocytes that line

intra-hepatic bile ducts. As cyst number and size increase, the associated fibrosis begins to affect liver function. Due to attention focused primarily on renal cyst formation, little is known about the processes that underlie hepatic biliary cyst development and maintenance. Like the renal cysts, the liver cysts arise from the proliferation of epithelial cells. The etiology of cyst formation may be similar in both the renal and the liver manifestations. For instance, it is known that the secretion of ions, predominately Cl⁻, into the cyst fluid is mediated by cAMP stimulation of the cystic fibrosis transmembrane regulator [2, 3]. However, the increased intracellular cAMP driving this process arises from different stimuli in renal and bile duct tissues. Likewise, the factors driving cellular proliferation and fibrotic changes may be similar or different in the two tissues. Identification and comparison of proteins present in both renal and hepatic biliary cyst fluids will facilitate the research endeavors, which are directed toward answering such questions.

The BALB/c-cpk mouse model, from which the fluid was collected, is a model of ARPKD. This model arises due to a mutation of the “cpk” gene. The cpk gene encodes the protein “cystin” that is expressed primarily in liver and kidney epithelial cells. BALB/c mice homozygous for a cpk

Correspondence: Dr. Frank A. Witzmann, Department of Cellular & Integrative Physiology, Indiana University School of Medicine, Biotechnology Research & Training Center 1345 West 16th Street, Room 308 Indianapolis, IN 46202, USA

E-mail: fwitzman@iupui.edu

Fax: +1-317-278-9739

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; AHSG, fetuin-A/α-2-HS glycoprotein; MMP, matrix metalloproteinase; PKD, polycystic kidney disease; SGP, sulfated glycoprotein; TRAF, tumor necrosis factor receptor-associated factor

mutation (BALB/c-cpk/cpk) rapidly develop cysts in various organs and die within 2–4 wk [4]. Heterozygous animals (BALB/c-cpk/+) have a relatively normal life span; however, as they age (12–18 months), they develop fluid-filled cysts arising from epithelial cells lining the intra-hepatic bile ducts. These animals used in the present study were all heterozygotes (BALB/c-cpk/+) because the homozygous animals only live for a few weeks and we could not therefore obtain sufficient fluid to analyze. There are no appropriate controls for this type of experiment because control animals do not have cysts.

The BALB/c-cpk/+ mice were bred at the Indiana University School of Medicine Laboratory Animal Resource Center and used under protocols approved by IACUC. Cystic BALB/c-cpk/+ mice (~18 months of age) in advanced disease stage were euthanized (150 mg pentobarbital *per* kilogram body weight) and perfused with PBS. Cyst fluid was collected by needle aspiration, immediately after the whole animal perfusion. One pooled sample was from multiple cysts of three mice, and the other three samples were from multiple cysts of individual mice. The collected cyst fluid was placed in D-Tube[®] dialyzing tubes (EMD Biosciences, San Diego, CA, USA) and de-salted overnight (8°C) against distilled water. Protein concentration was determined by Amido Black protein assay [5] and cyst fluid proteins were resuspended in HPLC-grade water to produce 1 µg/µL sample concentrations. Forty-five microgram of total protein was mixed with 5 µL of 1 M NH₄HCO₃ (final concentration 100 mM). Reduction and alkylation were carried out for 1 h at 37°C by adding an equal volume of a cocktail containing 2% iodoethanol, 0.5% triethylphosphine, and 97.5% ACN. The reaction mixture was evaporated to dryness by SpeedVac. The dried sample was reconstituted with 100 mM NH₄HCO₃ and digested with trypsin for 18 h at 37°C, and the digested protein mixture subsequently subjected to LC-MS/MS analysis. Each sample was injected three times. As the first protein composition study of this specific type sample, the intact samples have been analyzed to minimize potential loss of proteins resulting from fractionation or depletion of high-abundance proteins [6, 7].

Tryptic peptides were analyzed using a linear ion-trap mass spectrometer (Thermo-Finnigan) coupled with a Surveyor auto-sampler and MS HPLC system (Thermo-Finnigan). Peptides were injected onto a C18 microbore reversed-phase column (Zorbax 300SB-C18, 1 mm × 50 mm) and were eluted with a linear gradient from 5 to 45% ACN developed over 120 min at a flow rate of 50 µL/min. The data were collected in the “Triple-Play” (MS scan, Zoom scan, and MS/MS scan) mode with the ESI using a normalized collision energy of 35%. Dynamic exclusion settings were set to repeat count 1, repeat duration 30 s, exclusion duration 120 s, and exclusion mass width m/z 0.75 (low) and m/z 2.0 (high). The acquired data were searched against the IPI (International Protein Index) mouse database (ipi.MOUSE.v3.42) using SEQUEST (v. 28 rev. 12)

algorithms in Bioworks (v. 3.3). General parameters were as follows: peptide tolerance 2.0 AMU, fragment ion tolerance 1.0 AMU, enzyme limits set as “fully enzymatic cleaves at both ends”, and missed cleavage sites set at two. The searched peptides and proteins were validated by PeptideProphet [8] and ProteinProphet [9] in the Trans-Proteomic Pipeline (TPP, v. 3.3.0) (<http://tools.proteomecenter.org/software.php>).

Totally, 303 proteins with ≥90.00% (0.9000) confidence were identified by peptides with ≥90.00% confidence *via* TPP validation. The 123 representative proteins identified in hepatic cyst fluid are summarized in Table 1, where some proteins identified by the same peptides are placed into a single protein group. The protein group numbers, the numbers of proteins in each group, and the number of peptides used for protein identification are provided. The complete list of identified proteins and their identification information is available in the Supporting Information available online at the PROTEOMICS web site (www.proteomics-journal.com) and stored in the PRIDE database (<http://www.ebi.ac.uk/pride>, PRIDE Experiment Accession 9227).

It is interesting to compare protein identification in ARPKD liver cyst fluid with plasma, human liver cyst fluid, bile, and renal cyst fluid. Most of the 22 most abundant proteins comprising approximately 99% of the total protein mass in human plasma [10] appear in ARPKD liver cyst fluid, such as albumin, immunoglobulins, complement components, and apolipoproteins. No other previous publication has ever described a detailed proteomic analysis of the liver cyst fluid from a PKD rodent model or human. However, several cytokines and growth factors were reported in human ADPKD liver cyst fluid. These included angiogenin, interleukin-6, interleukin-8, monocyte chemoattractant protein-1, epithelial neutrophil attractant 78, and vascular endothelial growth factor [11]. These particular cytokines and growth factors were not identified in our study, possibly because their abundance is very low or because the BALB/c-cpk animals are a model of ARPKD rather than ADPKD. Bile is not an ideal control for liver cyst fluid, and its proteome has large differences from liver cyst fluid; however, they share some proteins that generally appear in plasma [12–15].

Comparing our results with the comprehensive analysis of protein composition in human renal cyst fluid by LC-MS/MS [16], the renal proteome of *jck* mice model characterized by two-dimensional gel electrophoresis combined with MS (MALDI-TOF/TOF) [17], and the proteome analysis of kidney cell membrane from an ADPKD mouse model by LC-MS/MS [18], it is found that both of liver and renal cyst fluid contain albumin, immunoglobulins, complement components, and some other plasma high-abundance proteins. Liver and renal cyst fluid share some proteins involved in cyst maintenance, growth, and structural formation, such as α-2-HS-glycoprotein, clusterin, hemopexin, serine-protease inhibitor family proteins, and

Table 1. The representative proteins identified with $\geq 90.00\%$ confidence (0.9000) by peptides with $\geq 90.00\%$ confidence from liver cyst fluid samples

IPI Number	Protein ID confidence	% Protein coverage	Protein common name	Protein group	No. of proteins in group	No. of peptides
IPI00845678	1.0000	16.3	60 kDa heat shock protein, isoform 2	1	2	2
IPI00473320	1.0000	13.6	Actin, β	2	3	3
IPI00885500	0.9932	4.3	Actin, γ 2, smooth muscle	3	1	1
IPI00874482	1.0000	13.6	Actin, γ , cytoplasmic 1	4	3	3
IPI00266875	1.0000	8.5	Actin, smooth muscle γ	5	9	2
IPI00136929	0.9999	7.2	Actin-like protein, γ	6	1	2
IPI00131695	1.0000	55.9	Albumin precursor	7	1	46
IPI00118130	0.9904	4.3	α -1-Acid glycoprotein 1	8	2	1
IPI00406302	1.0000	24.3	α -1-Antitrypsin 1-1 precursor	9	3	6
IPI00129755	1.0000	25.9	α -1-Antitrypsin 1-2 precursor	10	1	11
IPI00123924	1.0000	21.3	α -1-Antitrypsin 1-4 precursor	11	1	7
IPI00127352	0.9410	2.9	α -1-Microglobulin/bikunin precursor	12	1	1
IPI00118924	1.0000	5.7	α -2-Antiplasmin precursor	13	1	2
IPI00877275	0.9992	4.2	α -2-Glycoprotein 1, zinc binding	14	2	1
IPI00128249	1.0000	36.2	α -2-HS-glycoprotein precursor	15	1	7
IPI00624663	1.0000	4.2	α -2-Macroglobulin precursor	16	1	5
IPI00775996	0.9374	5.1	Amylase 2, pancreatic, similar to	17	3	1
IPI00136642	1.0000	15.5	Antithrombin-III precursor	18	1	4
IPI00877236	1.0000	22.7	Apolipoprotein A-I	19	2	5
IPI00869381	1.0000	14.7	Apolipoprotein A-II	20	2	1
IPI00775913	1.0000	16.5	Apolipoprotein A-IV	21	2	3
IPI00323571	1.0000	16.7	Apolipoprotein E, precursor	22	1	4
IPI00648243	1.0000	26.6	Apolipoprotein H	23	3	3
IPI00468481	1.0000	9.6	ATP synthase subunit β	24	1	3
IPI00130950	0.9989	5.9	Betaine-homocysteine S-methyltransferase 1	25	2	1
IPI00869393	0.9999	10.8	Catalase	26	3	1
IPI00113517	0.9999	4.7	Cathepsin B precursor	27	1	1
IPI00874570	1.0000	17.3	Ceruloplasmin	28	3	13
IPI00315696	0.9924	5.7	Chymotrypsinogen B precursor	29	1	1
IPI00320420	1.0000	31.7	Clusterin precursor	30	2	18
IPI00759878	1.0000	17.3	Complement component 3	31	1	22
IPI00323624	1.0000	14.3	Complement component 3, isoform long	32	1	17
IPI00672438	0.9999	1.5	Complement component 4, similar to	33	5	1
IPI00230718	0.9999	2.1	Complement component 9	34	1	1
IPI00130010	0.9999	13.2	Complement component factor H	35	3	1
IPI00114065	1.0000	3.8	Complement factor B	36	1	1
IPI00224671	0.9958	3.5	Complement factor D precursor, isoform 2	37	2	1
IPI00752688	0.9992	13.2	Complement factor H-related protein	38	5	1
IPI00320675	0.9999	2.2	Complement factor I, precursor	39	2	1
IPI00123744	0.9960	10.8	Cystatin C precursor	40	2	1
IPI00480452	0.9634	1.7	Death-inducing protein	41	3	1
IPI00875553	0.9120	1.2	DIP2 disco-interacting protein 2 homolog C	42	1	1
IPI00228548	0.9999	7.0	Enolase, β	43	6	1
IPI00411099	0.9999	2.6	Epidermal growth factor receptor, isoform 2	44	2	1
IPI00845610	0.9436	2.9	Fetuin, β isoform 2	45	3	1
IPI00885793	0.9998	2.9	Fibrinogen, α	46	2	1
IPI00279079	1.0000	12.9	Fibrinogen, β chain precursor	47	1	4
IPI00122312	1.0000	12.6	Fibrinogen, γ	48	1	3
IPI00652813	1.0000	2.6	Fibronectin 1	49	3	2
IPI00122487	0.9963	7.3	Forkhead-related transcription factor 10	50	1	1
IPI00127206	0.9685	7.4	Fructose-bisphosphate aldolase B	51	1	1
IPI00469114	1.0000	54.9	Hemoglobin, α adult chain 1	52	1	9
IPI00553333	1.0000	59.9	Hemoglobin, β adult major chain	53	1	5
IPI00316491	1.0000	54.1	Hemoglobin, β adult minor chain	54	2	5
IPI00845802	1.0000	28.2	Hemoglobin, subunit α	55	2	3

Table 1. Continued

IPI Number	Protein ID confidence	% Protein coverage	Protein common name	Protein group	No. of proteins in group	No. of peptides
IPI00762198	1.0000	44.9	Hemoglobin, subunit β -1	56	1	5
IPI00831055	1.0000	23.8	Hemoglobin, subunit β -1	57	1	2
IPI00885376	1.0000	13.0	Hemoglobin, subunit β -1, fragment	58	2	2
IPI00877345	1.0000	30.7	Hemopexin	59	2	17
IPI00223444	0.9451	10.6	Heterogeneous nuclear ribonucleoproteins C1/C2, isoform 3	60	6	1
IPI00322304	0.9999	5.0	Histidine-rich glycoprotein	61	1	1
IPI00845732	1.0000	14.8	Immunoglobulin epsilon, similar to	62	7	6
IPI00854003	0.9999	17.0	Immunoglobulin heavy chain	63	7	1
IPI00605486	1.0000	17.0	Immunoglobulin heavy chain, 1A	64	7	3
IPI00468055	1.0000	6.6	Immunoglobulin heavy chain, 6	65	4	2
IPI00460350	0.9981	3.1	Immunoglobulin heavy chain, γ	66	3	1
IPI00556780	0.9999	4.8	Immunoglobulin heavy chain, γ	67	8	1
IPI00881239	0.9999	14.5	Immunoglobulin heavy chain, γ	68	2	1
IPI00475246	1.0000	6.8	Immunoglobulin heavy chain, γ 1	69	3	2
IPI00136925	0.9994	9.4	Immunoglobulin J chain precursor	70	1	1
IPI00553402	0.9982	12.7	Immunoglobulin κ chain, variable 21	71	21	2
IPI00831490	0.9453	13.8	Immunoglobulin κ chain, variable 33	72	2	1
IPI00660980	0.9999	18.9	Immunoglobulin λ chain, variable 1	73	3	1
IPI00312711	1.0000	4.6	Inter α -trypsin inhibitor, heavy chain 4	74	1	3
IPI00881701	0.9999	1.4	Inter α -trypsin inhibitor, heavy chain 4	75	2	1
IPI00230702	0.9999	2.6	Intercellular adhesion molecule 1 precursor, isoform 2	76	2	1
IPI00124499	0.9999	3.6	Keratin	77	1	2
IPI00311493	0.9834	3.5	Keratin	78	5	1
IPI00330480	0.9999	5.9	Keratin	79	6	1
IPI00347110	0.9990	7.8	Keratin	80	1	2
IPI00462140	0.9956	5.4	Keratin	81	1	2
IPI00875096	0.9948	3.7	Keratin	82	2	2
IPI00876179	0.9834	5.1	Keratin	83	2	1
IPI00625729	1.0000	4.9	Keratin 1	84	1	4
IPI00470126	1.0000	5.9	Keratin 5	85	2	1
IPI00755181	1.0000	9.1	Keratin, complex 1	86	3	4
IPI00463282	0.9948	3.3	Keratin, fragment	87	1	1
IPI00856249	1.0000	11.8	Kininogen 1	88	5	3
IPI00129250	1.0000	7.9	Leucine-rich α -2-glycoprotein precursor	89	1	2
IPI00115941	1.0000	20.5	Lipocalin 2	90	1	2
IPI00626863	1.0000	13.5	Lipocalin 2	91	1	2
IPI00138342	1.0000	8.7	Liver carboxylesterase N precursor	92	1	4
IPI00648966	1.0000	15.0	Major urinary protein 1	93	10	2
IPI00850482	0.9999	8.9	Major urinary protein 1, similar to	94	9	1
IPI00121279	1.0000	6.1	Mesothelin, precursor	95	1	2
IPI00553387	0.9561	3.8	Metabotropic glutamate receptor 5	96	2	1
IPI00308990	1.0000	13.7	Monocyte differentiation antigen CD14 precursor	97	1	3
IPI00849570	0.9844	1.5	Murinoglobulin 1, similar to	98	2	2
IPI00123223	1.0000	3.6	Murinoglobulin-1 precursor	99	1	2
IPI00420891	1.0000	2.1	Murinoglobulin-4 precursor	100	2	3
IPI00309133	1.0000	16.7	Osteopontin precursor	101	2	1
IPI00323644	0.9175	1.4	Phosphoribosylglycinamide formyltransferase	102	2	1
IPI00322936	0.9984	1.6	Plasminogen precursor	103	1	1
IPI00129754	0.9999	5.6	Podocalyxin	104	2	1
IPI00230257	0.9863	2.4	Polyhomeotic-like protein1, isoform 3	105	5	1
IPI00310059	1.0000	7.7	Polymeric immunoglobulin receptor, precursor	106	1	3
IPI00122815	0.9999	5.9	Prolyl 4-hydroxylase, β polypeptide	107	2	1
IPI00264822	0.9053	2.3	Protein sel-1 homolog 2 precursor	108	1	1

Table 1. Continued

IPI Number	Protein ID confidence	% Protein coverage	Protein common name	Protein group	No. of proteins in group	No. of peptides
IPI00121582	0.9131	2.1	Regulatory factor X, 3	109	3	1
IPI00119202	0.9804	16.7	S100 calcium-binding protein A11	110	4	1
IPI00222556	0.9999	12.4	S100 calcium-binding protein A9	111	1	1
IPI00458284	1.0000	9.9	Serine peptidase inhibitor	112	2	2
IPI00116105	1.0000	13.6	Serine peptidase inhibitor, clade A, member 6	113	2	4
IPI00881377	1.0000	13.5	Serine peptidase inhibitor, clade G, member 1	114	3	2
IPI00475157	1.0000	26.9	Serine peptidase inhibitor, clade A, 1b	115	1	6
IPI00131830	1.0000	35.6	Serine protease inhibitor A3K precursor	116	1	19
IPI00135635	0.9703	18.9	Serine protease inhibitor A3M precursor	117	1	7
IPI00139788	1.0000	31.7	Serotransferrin precursor	118	1	25
IPI00471080	1.0000	3.9	Serotransferrin precursor	119	1	2
IPI00474463	0.9923	1.3	TRAF7	120	2	1
IPI00127560	1.0000	44.2	Transthyretin precursor	121	1	6
IPI00126184	1.0000	19.2	Vitamin D-binding protein precursor	122	2	7
IPI00129240	0.9999	3.8	Vitronectin precursor	123	1	1

vitronectin, which have been discussed in the renal cyst fluid proteome study by the investigators [16]. The major urinary protein identified in *jck* mouse kidney also appears in ARPKD mouse liver cyst fluid. Most of proteins identified in ARPKD mouse liver cyst fluid are not membrane proteins. Furthermore, some proteins are unique in liver cyst fluid including actin, α -2-macroglobulin, lipocalin 2 (neutrophil gelatinase-associated lipocalin), liver carboxylesterase N, mesothelin, monocyte differentiation antigen CD14 (CD14), murinoglobulin-1 (α -1-inhibitor III), murinoglobulin 4, osteopontin (secreted phosphoprotein 1), polymeric immunoglobulin receptor, and tumor necrosis factor receptor-associated factor 7 (TRAF7), some of which will be discussed below.

The fluid from liver cysts has been shown to contain factors that initiate and maintain the cysts by enhancing cellular proliferation and also, more acutely, by stimulating secretory processes which increase cystic fluid and encourage cyst expansion [19, 20]. Proteomic studies can provide insight into the identity of these components of the cyst fluid. The cystic proteome contains many elements, which are congruent with the fibrosis noted in human as well as animal models. Transcriptome analysis of a rat model of PKD has indicated the up-regulation of a relatively large number of genes, which are involved in extracellular matrix formation [21]. In agreement with these studies, some of the products of these genes are found in cyst fluid, notably fibronectin, fibrinogen, and serine-cysteine proteinase inhibitors. Additionally, other matrix proteins or proteins involved in the metabolism of the extracellular matrix occur in biliary cyst fluid. These include vitronectin, α -2-macroglobulin, and osteopontin (secreted phosphoprotein 1). In cultured cystic human biliary epithelial cells matrix metalloproteinase (MMP) activity is increased compared with the

normal cholangiocytes [22]. MMPs are a large family of extracellular proteases, which degrade extracellular matrix and, therefore, are involved in processes as diverse as inflammation, wound healing, and carcinogenic transformation. Though MMP proteins were not detected, α -2-macroglobulin, an inhibitor of MMP [23] previously found to be elevated in fibrotic livers [24], was identified. Also identified were cyst fluid proteins related to cell differentiation or maturation. Because of the importance of extracellular matrix in cellular differentiation, many of these proteins overlap with proteins of the extracellular matrix. For instance, fibronectin and vitronectin have been shown to regulate cellular differentiation and development [25].

Other proteins associated with differentiation and proliferation are fetuin-A/ α -2-HS glycoprotein (AHSG) and sulfated glycoprotein-2 (SGP-2, clusterin). AHSG is expressed in mouse limb buds and brain only at specific stages of development. In studies performed by Yang *et al.*, mouse liver AHSG/fetuin mRNA was shown to be expressed at low levels at about 12 days gestation but increased and peaked at approximately 3 months after birth. Subsequently this level decreases dramatically in both mice and humans [26]. The presence of AHSG in cyst fluid from animals over a year of age suggests a return to an immature state of cellular differentiation. SGP-2 or clusterin, is a protein associated with collecting duct cell immaturity [27], and presence of this protein in urine has been associated with tubular damage and with the presence of PKD. Urinary levels of SGP-2/clusterin were used to follow the amelioration of renal failure by epidermal growth factor treatment in C57BL/6J cpk/cpk model of ARPKD [28]. Urinary clusterin levels have also been used as a marker of tubular damage in rat models of PKD and renal ischemia [29]. Clusterin was found in cyst fluid thus indicating that this protein may also

serve as a marker of cell damage and/or immaturity of the biliary cholangiocytes.

Interstitial inflammation has also been correlated with PKD disease progression and fibrosis. In the Han:SPRD rat model of ADPKD, increased levels of osteopontin have been correlated with an increased accumulation of macrophages in the interstitium of cystic kidneys [30]. The appearance of osteopontin in the hepatic cyst fluid suggests a similar mechanism in this tissue. The cyst fluid also contains proteins that have previously been used as markers for acute kidney injury [31] including fetuin-A, and lipocalin 2 (neutrophil gelatinase-associated lipocalin). Monocyte differentiation antigen CD14 (CD14) can act as a receptor that binds bacterial LPS, triggering inflammatory responses [32]. Murinoglobulin-1 (α -1-inhibitor III) is a negative acute-phase protein, whose plasma concentrations of are decreased during an inflammatory response [33]. The presence of these proteins in the hepatic cyst fluid is indicative of bile duct injury, which is consistent with the inflammatory response.

Some proteins identified in ARPKD mouse liver cyst fluid have not been reported to be associated with the differentiation and proliferation of cyst. However, their functions reveal that they may be involved in the initiation and development of liver cyst. Mesothelin plays a role in cellular adhesion [34]. The overexpression of TRAF7, one of the TRAF protein family members, induces caspase-dependent apoptosis through distinct domains [35]. Actin, one of the main proteins of muscle and cytoskeleton, exists as a variety of highly conserved isoforms whose distribution in vertebrates is tissue-specific [36]. However, it was

discovered in ARPKD mouse liver cyst fluid. The mechanism is unclear but this finding is interesting.

To find potential links between the identified proteins and particular signaling or biochemical pathways, pathway analysis was generated through the use of Ingenuity Pathways Analysis (Ingenuity Systems[®], www.ingenuity.com). Proteins were uploaded onto a global molecular network developed from information contained in the Ingenuity Pathways Knowledge Base. Networks of these proteins were then algorithmically generated based on their connectivity. All relationships are supported by at least one reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base [37]. In the top four networks list, the inflammatory disease network ranked No. 1, and had a much better score than the others. Figure 1 shows a graphical representation of the molecular relationships between proteins. Cystic change is invariably associated with variable degrees of inflammatory infiltrate. Pro-inflammatory cytokines have been identified in ADPKD cyst fluid. The treatment of a PKD rat model with an anti-inflammatory drug markedly reduces cystic change [38]. Our pathway analysis of proteins from ARPKD mouse liver cyst fluid confirms that inflammatory response plays a role in mediating cyst formation.

The proteomic analysis of hepatic cyst fluid protein function conducted here represents the first of its kind, and is based on analogy and theoretical considerations. Nonetheless, these data provide the basis for the formulation of testable postulates for the role of identified proteins in cyst development and maintenance. Collectively, we have demonstrated an array of proteins whose presence is consistent with previously observed structural analyses and

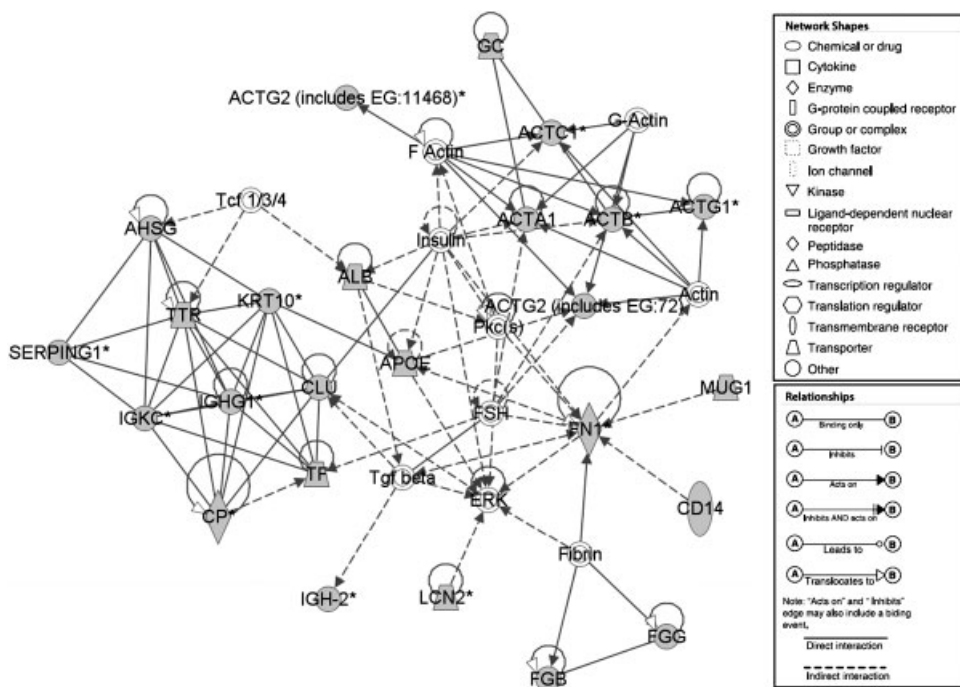


Figure 1. Pathway analysis of proteins identified in ARPKD mouse liver cyst fluid. The inflammatory disease network is shown in a graphical representation of the molecular relationships between proteins. Proteins are represented as nodes, and the biological relationship between two nodes is represented as a line. Nodes and lines are displayed using various shapes that represent the functional class of proteins and different relationships. A gray node indicates that this protein is identified in liver cyst fluid.

provide a substantial number of target proteins whose role in cyst development and maintenance are of great interest to the PKD research community and can be examined in future studies.

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