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The biomarker enriched proteome of autosomal dominant polycystic kidney disease cyst fluid

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by the development of numerous fluid-filled cysts in the kidneys of patients. We recently published our description of the proteome of renal cyst fluid in ADPKD. As a follow-up experiment, we hypothesized that the protein-bound subfraction consists of molecules of mechanistic or diagnostic interest in ADPKD. Using a manual biomarker enrichment kit, we have identified 44 distinct proteins in human cyst fluid.

Keywords

Albuminome; Biomarker; Carrier protein; Complement; MS

Mutations in the gene that codes for the protein polycystin result in the multi-organ disease Autosomal Dominant Polycystic Kidney Disease (ADPKD). This genetic disorder causes renal failure in adults secondary to polycystic kidneys and associated renal fibrosis [1]. We are characterizing the proteome of cyst fluid in polycystic kidney disease (PKD) to define proteins of mechanistic or diagnostic importance in this disease.

Our previously published experiments [2] with human renal cyst fluid identified over 390 proteins. A higher yield was obtained using the ProteoPrep® 20 (Sigma-Aldrich) immunodepletion kit to remove albumin (ALB) and 19 additional high abundance proteins present in the cyst fluid, thereby enhancing the ability to detect lower abundance proteins. Since ALB and other carrier molecules bind proteins and peptides in the serum [3] we hypothesized that the protein-bound subfraction of renal cyst fluid would contain proteins of pathophysiologic interest in PKD. A recent paper by Gundry [4] highlights the plethora of proteins that bind to carrier molecules in human sera. Using the ProXPRESSION™ Biomarker

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Manual Enrichment kit (PerkinElmer), we sought to define the protein-bound fraction of cyst fluid. The kit contains Cibacron Blue, a nonselective ALB-binding dye. Although the enrichment kit is designed to bind ALB, we understand from the manufacturer (Vivascience) that the column may bind other proteins to a lesser degree, therefore we describe our identified proteome as “biomarker enriched” rather than “ALB-bound” [5].

Our cyst samples were identical to the four samples (CF02–CF05) used in the previously published experiment [2]. These de-identified human samples are cyst fluid collections that were managed according to the bioethical recommendations of the Institutional Review Board. The cyst fluid was aspirated postoperatively from excised kidneys in patients with end-stage renal disease.

Since the binding capacity of the Vivapure™ Blue column in the ProXPRESSION™ kit is 4 mg, we collected a calculated volume of cyst fluid that would result in approximately 3 mg of protein entering the column. Cyst fluid was centrifuged and the calculated volume of supernatant was diluted 1:10 with binding buffer. After loading the diluted supernatant onto a Vivapure™ Blue spin column, the column was washed three times with binding buffer to remove unbound proteins. The column contains a patented chromatography membrane. This membrane combined with the proprietary buffer and elution chemistries reproducibly elutes peptides and proteins bound to carrier proteins. To elute the peptides, 200 μ L of elution buffer was introduced onto the Vivapure™ columns. The column was centrifuged at $250 \times g$ for 2 min and the elution step was repeated *per* the ProXPRESSION™ kit instruction manual.

To prepare samples for analysis by tandem MS, we performed a Bradford assay to determine the concentration of each eluent. Using 6 M urea we diluted the sample to reach 200 μ L of total volume, resulting in an approximate protein concentration of 500 μ g/mL *per* sample. Next 200 μ L of a reduction/alkylation cocktail was introduced into each sample; this cocktail contains ACN, iodoethanol, and triethylphosphine. After 90 min incubation the samples were vacuum dried overnight. Each sample was resuspended in NH_4HCO_3 and 150 μ L of a 20 μ g/mL sequence grade trypsin (Princeton Separations) solution was added prior to incubation at 37°C. After 3 h another 150 μ L of trypsin was added and the sample incubated overnight.

The samples were analyzed in replicate using a Thermo-Finnigan linear ion-trap mass spectrometer with a Surveyor autosampler and MS HPLC system (Thermo-Finnigan). The scan was completed in “Triple Play” (MS scan, Zoom scan, and MS/MS scan) mode. The acquired data was searched against the international protein index (IPI) human database (ipi.HUMAN.v3.34) using SEQUEST (v.28 rev.12) algorithms in Bioworks (v.3.3). Parameters for the search were set to: fragment ion tolerance 1.0 amu, peptide tolerance 2.0 amu, enzyme limits set as “fully enzymatic – cleaves at both ends”, and cleavage sites set at 2. These search results were validated using PeptideProphet [5] and ProteinProphet [6] in the trans-proteomic pipeline (TPP, v. 3.3.0) (<http://tools.proteomecenter.org/software.php>). The identified proteins were entered into the Pathway Studio© 5.0 software for analysis (Fig. 1).

We identified 142 proteins from the biomarker-enriched subfraction that had at least 90% confidence *via* TPP. Of these, 44 proteins were found to be (i) common to at least two cysts and (ii) distinct, meaning that each identified protein had at least one unique peptide (Table 1). The table outlines how many cysts each protein was identified in, ranging from 2/4–4/4 cysts. Ten of these proteins (bold font) were identified only in the biomarker-enriched sample, suggesting a highly protein-bound state in cyst fluid. We hypothesize that many of the proteins identified in both experiments are fluctuating between a protein-bound and unbound state. A Supporting Information Table lists these 44 proteins along with the peptide sequences used for identification and their probability. Proteins of interest include apolipoprotein A-1 (APOA-1), complement C3, pigment epithelium-derived factor (PEDF), along with many others. APOA-1

has been found to be overly expressed in previous studies of PKD in rodent models [6]. Complement C3 and other innate immunity components have recently been implicated in the pathogenesis of cysts in ARPKD, and the authors believe there is a similar pathogenetic mechanism in ADPKD [7]. PEDF appears to be highly protein-bound and its presence in all sample injections in this study has prompted us to propose experiments studying this protein's effect on cyst growth *in vitro*. The Supporting Information Table can be found on our website (<http://anatomy.iupui.edu/mason/pkd-proteomics.htm>) or at the online repository PRIDE (www.ebi.ac.uk/pride).

Figure 1 displays the results of a Pathway Studio® analysis (www.ariadnegenomics.com) conducted to assess potential functional significance of the detected proteins. Pathway Studio® finds common regulators and associates pathway components with like-behaving biological entities and processes. Protein identification numbers (Swiss-Prot) are submitted and a flow diagram is produced demonstrating protein interactions and pathways of interaction. It is noted that APOA-1 and antithrombin III (SERPINC1) are known to complex with ALB [4]. The most notable interactions are between plasminogen (PLG) and several other identified proteins. These interactions do not appear to be specific to the pathology of PKD.

We compared our renal cyst subfraction to the ALB-enriched serum sample as defined by Gundry *et al.* [4]. Our study identified 44 whereas Gundry identified 120 distinct proteins. Interestingly, our sample contains eight unique complement components (redundant isoforms and precursors excluded) as opposed to seven in the Gundry serum sample (clusterin is included as a complement component). An enrichment of these innate immunity proteins in cyst fluid relative to the Gundry serum sample is interesting. We hypothesize that this enrichment of complement components in our subfraction is characteristic of PKD, and corroborates the recent finding of Mrug *et al.* in the *cpk* mouse model of recessive polycystic disease. In that study, complement C3, adipsin (factor D), clusterin, and ten other complement component genes are overexpressed in cystic renal tissue [7]. We have confirmed the presence of complement C3 and factor D in this subfraction of ADPKD cyst fluid.

In our previous paper [2], we had hypothesized that epidermal growth factor (EGF) would be identified in the biomarker enriched subfraction of cyst fluid. However, EGF was not identified in this experiment. A likely explanation lies in the severity of the renal disease in our patient samples. Since these cyst fluid samples were obtained at the time of dialysis initiation, all surgically excised kidneys were necessarily from end-stage disease patients. Weinstein *et al.* found low urinary excretion of EGF in ADPKD patients at an early stage in the disease [8]. It has been shown that urine EGF virtually disappears in chronic renal failure patients [9].

This study follows our characterization of the renal cyst fluid proteome [2]. We have identified the biomarker-enriched proteome of ADPKD fluid. We identified 44 distinct proteins; including molecules of mechanistic interest, such as APOA-1, PEDF, and complement C3. The relative enrichment of protein-bound complement components highlights a difference between the serum and cyst fluid proteomes. Future studies defining the role of innate immunity components in cystogenesis and cyst growth could lead to therapeutic intervention in PKD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ADPKD	autosomal dominant polycystic kidney disease
ALB	albumin
APO	apolipoprotein
EGF	epidermal growth factor
IPI	international protein index
PEDF	pigment epithelium-derived factor
PKD	polycystic kidney disease
PLG	plasminogen
TPP	trans-proteomic pipeline

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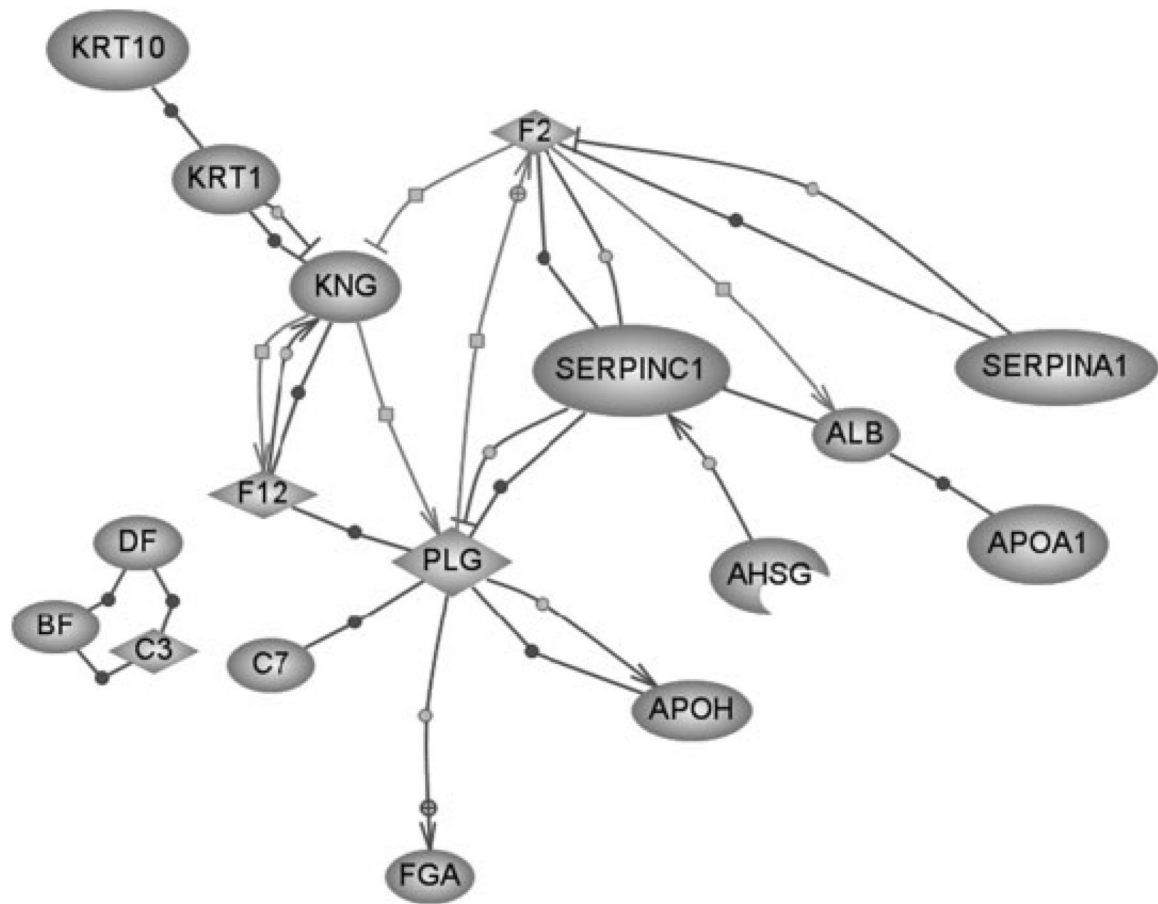


Figure 1.

Results of a Pathway Studio® analysis of direct relationships between the components of the protein-bound fraction of renal cyst fluid. See text for interpretation. [AHSG = α -2-HS-glycoprotein, ALB, APOA-1, APOH = apolipoprotein H, BF = complement factor B, C3 = complement component 3, C7 = complement component 7, DF = complement factor D, F2 = coagulation factor II, F12 = coagulation factor XII (Hageman factor), FGA = fibrinogen, alpha polypeptide, KNG = K-kininogen, KRT1 = type II keratin Kb1, KRT10 = keratin 10, PLG, SERPINA1 = serine (or cysteine) proteinase inhibitor, clade A (antitrypsin), member 1, SERPINC1 = serine (or cysteine) peptidase inhibitor, clade C (antithrombin), member 1].

Table 1

Protein-bound proteins that were found to be common to at least two cysts

IPI number	Common name of protein	TPP confidence	Number of matching peptides	Percent coverage (%)
IPI00745872	Serum ALB precursor, isoform 1*	1.0000	94	75.0
IPI00298828	β -2-glycoprotein 1 precursor*	1.0000	18	47.8
IPI00816555	IGLV2-14 protein*	1.0000	2	46.2
IPI00022488	Hemopexin precursor*	1.0000	21	45.5
IPI00014048	Ribonuclease pancreatic precursor*	1.0000	3	43.6
IPI00845354	IGKC protein*	1.0000	10	40.6
IPI00430808	Immunoglobulin light chain*	1.0000	6	37.0
IPI00784985	Putative uncharacterized protein*	1.0000	6	37.0
IPI00026314	Gelsolin precursor, isoform 1*	1.0000	20	35.7
IPI00021841	APO A1 precursor*	1.0000	7	33.5
IPI00019580	PLG precursor*	1.0000	16	33.3
IPI00399007	Putative uncharacterized protein DKFZp686I04196*	1.0000	15	32.9
IPI00472345	IGHG3 protein*	1.0000	8	31.3
IPI00006114	PEDF precursor*	1.0000	11	26.8
IPI00165972	Complement factor D preproprotein*	1.0000	4	20.4
IPI00032293	Cystatin-C precursor*	1.0000	3	19.2
IPI00377087	Gelsolin precursor, isoform 2+	1.0000	2	18.6
IPI00022371	Histidine-rich glycoprotein precursor*	1.0000	6	17.0
IPI00019581	Coagulation factor XII precursor*	1.0000	6	16.8
IPI00011264	Complement factor H-related protein 1 precursor*	1.0000	4	16.7
IPI00152331	Metastasis suppressor protein 1, isoform 1§	0.9702	1	15.2
IPI00829877	IGL@ protein*	1.0000	2	15.0
IPI00022420	Plasma retinol-binding protein precursor*	1.0000	2	12.1
IPI00022426	AMBP protein precursor*	1.0000	3	11.1
IPI00019591	Complement factor B precursor (Fragment), isoform 1*	1.0000	5	10.7
IPI00032328	HMW of Kininogen-1 precursor, isoform+	1.0000	2	10.3
IPI00021885	Fibrinogen α -chain precursor, isoform 1*	1.0000	6	9.9
IPI00218508	Complement factor B precursor (Fragment), isoform 2+	1.0000	3	9.7
IPI00029739	Complement factor H precursor, isoform 1+	1.0000	7	9.4
IPI00011252	Complement component C8 α -chain precursor§	1.0000	3	7.7
IPI00294395	Complement component C8 β -chain precursor+	1.0000	3	6.9
IPI00218999	Complement factor H precursor, isoform 2§	1.0000	3	6.9
IPI00021145	Dual specificity protein phosphatase CDC14A, isoform 1+	0.9963	1	6.6
IPI00022431	α -2-HS-glycoprotein precursor*	0.9999	1	5.4
IPI00032179	Antithrombin III variant*	0.9956	1	5.4
IPI00009865	Keratin, type I cytoskeletal 10§	1.0000	2	4.7
IPI00555812	Vitamin D-binding protein precursor*	1.0000	2	4.6
IPI00220327	Keratin, type II cytoskeletal 1+	0.9997	2	4.3
IPI00296608	Complement component C7 precursor§	1.0000	1	3.1

IPI number	Common name of protein	TPP confidence	Number of matching peptides	Percent coverage (%)
IPI00299568	Cytochrome P450 2A6+	0.9993	2	2.9
IPI00019568	Prothrombin precursor (Fragment)+	1.0000	1	2.7
IPI00553177	α -1-Antitrypsin precursor*	0.9865	1	2.2
IPI00783987	Complement C3 precursor (Fragment)*	0.9447	1	1.3
IPI00784368	Glucosaminyl N-deacetylase/N-sulfotransferase§	0.9746	1	0.8

Proteins identified only in the biomarker enriched samples *versus* depleted cyst fluid results from our prior experiment [2] are in bold font. Common names of proteins are followed by a symbol to recognize the number of cysts the protein was identified in: 4/4 cysts (*), 3/4 cysts (+) and 2/4 cysts (§). Each protein is distinct, meaning that each identified protein had at least one unique peptide. Peptides and proteins were identified by SEQUEST and validated *via* the TPP *via* PeptideProphet and ProteinProphet. Only those identified with > 90% confidence is listed. IPI accession numbers are linked to the common names.