(51) International Patent Classification:
G01N 33/68 (2006.01)

(21) International Application Number:
PCT/US20 16/062075

(22) International Filing Date:
15 November 2016 (15.11.2016)

(24) Filing Language:
English

(25) Publication Language:
English

(30) Priority Data:
62/257,089 18 November 2015 (18.11.2015) US


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Declarations under Rule 4.17:
— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(i))
— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(ii))
— of inventorship (Rule 4.17(iv))

Published:
— with international search report (Art. 21(3))
— with sequence listing part of description (Rule 52(a))

(54) Title: BIOMARKER OF POLycystic Kidney Disease AND USES THEREOF

Figure 17

(57) Abstract: Provided herein are methods for determining the efficacy of treatment for polycystic kidney disease (PKD) in a patient, diagnosing PKD in a patient, staging PKD in a patient, and monitoring PKD in a patient. These methods include determining a single or multiple levels of AMBP. Also provided are kits that include an antibody specifically binds to AMBP protein and at least one antibody that specifically binds to an additional marker of PKD.
BIOMARKER OF POLYCYSTIC KIDNEY DISEASE
AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No. 62/257,089, filed November 18, 2015, the entire contents of this application is herein incorporated by reference.

TECHNICAL FIELD

This invention relates to methods of molecular medicine and molecular biology.

BACKGROUND

Polycystic kidney disease (PKD) is a common genetic disorder characterized by the formation of fluid-filled epithelial-lined cysts in the kidneys of patients over time (Park et al., BMB Reports 44:359-368, 2011). The cysts in a PKD patient can increase in size and number over the decades, and displace and destroy adjacent renal parenchyma, which can ultimately lead to end-stage renal disease in the patient (Chapin et al., J. Cell Biol. 191:701-710, 2010). Multiple mechanisms have been shown to contribute to PKD, including increased proliferation and apoptosis, in addition to loss of differentiation and polarity (Belibi et al, Expert. Opin. Invest. Drugs 19:315-328, 2010). Many end-stage PKD patients depend on transplantation or hemodialysis to attenuate renal failure (Park et al, 2011; supra).

There are two types of PKD: autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). In the year 2006, about 500,000 people were diagnosed as having PKD in the U.S., with ADPKD affecting about 1 person out of 500 to 1,000 people, and ARPKD affecting about 1 person out of 20,000 to 40,000 people. ADPKD is the most common inherited disorder of the kidneys and accounts for -5% of the end-stage renal disease patients in the U.S. (Pei et al., Adv. Chronic Kidney Dis. 17:140-152, 2010).

SUMMARY

The present invention is based, at least in part, on the discovery that levels of a-1-microglobulin/bikunin precursor (AMBP) are elevated in samples including a biological fluid (e.g., a urine sample) or kidney tissue (e.g., a kidney biopsy sample) from patients having PKD, are increased in samples including a biological fluid (e.g., a urine sample) or kidney...
tissue (e.g., a kidney biopsy sample) from patients with later stages of PKD as compared to the levels in patients having earlier stages of PKD, and are decreased in samples including a biological fluid (e.g., a urine sample) or kidney tissue (e.g., a kidney biopsy sample) from PKD subjects administered a therapeutically effective treatment of PKD. In view of this discovery, provided herein are methods for determining the efficacy of a treatment for PKD in a patient, diagnosing PKD in a patient, staging PKD in a patient, and monitoring PKD in a patient that include determining a level of AMBP.

Provided herein are methods of determining the efficacy of treatment for polycystic kidney disease (PKD) in a patient that include: (a) providing a first sample including a biological fluid obtained from a PKD patient; (b) determining a level of a-1-microglobulin/bikunin precursor (AMBP) in the first sample; (c) administering a PKD treatment to the patient; (d) providing a second sample including a biological fluid from the patient after step (c) and determining a level of AMBP in the second sample; and (e) identifying the administered treatment as effective if the level in the second sample is lower than the level in the first sample. In some embodiments of these methods, the PKD patient has autosomal dominant PKD. In some embodiments of these methods, the PKD patient has autosomal recessive PKD. In some embodiments of these methods, the first and second samples include urine.

In some embodiments of these methods, the PKD treatment includes a glucosyl ceramide synthase (GCS) inhibitor. In some embodiments of these methods, the GCS inhibitor is selected from the group of: (S)-quinulclidin-3-yl (2-(4′-(2-methoxyethoxy)-[I, 1′-biphenyl]-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinulclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinulclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinulclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinulclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinulclidin-3-
ylopiperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; quinuclidin-3-yl (2-(4'-fluoro-[1,1'-biphenyl]-3-yl)propan-2-yl)carbamate; and carbamic acid, N-[1-[2-(4-fluorophenyl)-4-thiazolyl]-1′-methylene]-, (3S)-1-azabicyclo[2.2.2]oct-3-yl ester.

Some embodiments of these methods further include: (f) administering to the patient additional doses of GCS inhibitor if the treatment is identified as being effective. In some embodiments of these methods, the additional doses of GCS inhibitor include (S)-quinuclidin-3-yl(2-(4'-(2-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrinddin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[2.2.2]oct-3-yl ester.

In some embodiments of these methods, the PKD treatment includes a CDK inhibitor (e.g., R-roscovitine or S-CR8). Some embodiments of these methods further include: (f) administering to the patient additional doses of CDK inhibitor if the treatment is identified as being effective. In some embodiments of these methods, the additional doses of CDK inhibitor include R-roscovitine or S-CR8.

In some embodiments of these methods, determining the level of AMBP in (b) and (d) include determining the level of AMBP protein. In some embodiments of these methods, (b) and (d) include contacting the sample with an antibody that binds specifically to AMBP protein.
Also provided herein are methods of determining the stage of polycystic kidney disease (PKD) in a patient that include: (a) providing a sample including a biological fluid from a patient suspected of having PKD or identified as having PKD; (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the sample; and (c) determining the stage of PKD in the patient from the level. In some embodiments of these methods, the PKD is autosomal dominant PKD. In some embodiments of these methods, the PKD is autosomal recessive PKD. In some embodiments of these methods, the sample includes urine.

Some embodiments of these methods further include (d) administering a treatment for stage I, stage II, stage III, stage IV, or stage V PKD to a patient identified to have stage I, stage II, stage III, stage IV, or stage V PKD, respectively. Some embodiments of these methods further include: (d) imaging one or both kidney(s) in the patient after (c) to confirm the stage of PKD in the patient.

In some embodiments of these methods, determining the level of AMBP in (b) includes determining the level of AMBP protein. In some embodiments of these methods, (b) includes contacting the sample with an antibody that binds specifically to AMBP protein.

Also provided are methods of determining the stage of polycystic kidney disease (PKD) in a patient that include: (a) providing a sample including kidney tissue from a patient suspected of having PKD or identified as having PKD; (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the sample; and (c) determining the stage of PKD in the patient from the level. In some embodiments of these methods, the PKD is autosomal dominant PKD. In some embodiments of these methods, the PKD is autosomal recessive PKD.

Some embodiments of these methods further include: (d) administering a treatment for stage I, stage II, stage III, stage IV, or stage V PKD to a patient identified to have stage I, stage II, stage III, stage IV, or stage V PKD, respectively. Some embodiments of these methods further include: (d) imaging one or both kidney(s) in the patient after (c) to confirm the stage of PKD in the patient.

In some embodiments of these methods, determining the level of AMBP in (b) includes determining the level of AMBP protein. In some embodiments of these methods, (b) includes contacting the sample with an antibody that binds specifically to AMBP protein.

Also provided are methods of diagnosing polycystic kidney disease (PKD) in a patient that include: (a) providing a sample including a biological fluid from a patient suspected of having PKD; (b) determining a level of α-1-microglobulin/bikunin precursor
(AMBP) in the sample; and (c) identifying the patient as having PKD if the level is elevated as compared to a control level. In some embodiments of these methods, the PKD is autosomal dominant PKD. In some embodiments of these methods, the PKD is autosomal recessive PKD. In some embodiments of any of these methods, the sample includes urine.

Some embodiments of these methods further include: (d) administering a PKD treatment to the patient. In some embodiments of these methods, the PKD treatment includes a glucosyl ceramide synthase (GCS) inhibitor. In some embodiments of these methods, the GCS inhibitor is selected from the group of: (S)-quinuclidin-3-yl (2-(4′-(2-methoxyethoxy)-1,1′-biphenyl)-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(3-methylenecyclohexyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; quinuclidin-3-yl (2-(4′-fluoro-[1,1′-biphenyl]-3-yl)propan-2-yl)carbamate; and carbamic acid, N-[1-[2-(4-fluorophenyl)-4-thiazolyl]-1-methyl ethyl]-, (3S)-1-azabicyclo[2.2.2]oct-3-yl ester. In some embodiments of these methods, the PKD treatment includes a CDK inhibitor (e.g., R-roscovitine or S-CR8).

Some embodiments of these methods further include: (d) imaging one or both kidney(s) in the patient. In some embodiments of these methods, the control level is a threshold level or a level in a healthy subject or a population of healthy subjects.

In some embodiments of these methods, determining the level of AMBP in (b) includes determining the level of AMBP protein. In some embodiments of these methods, (b) includes contacting the sample with an antibody that binds specifically to AMBP protein.
Also provided herein are methods of diagnosing polycystic kidney disease (PKD) in a patient that include: (a) providing a sample including kidney tissue from a patient suspected of having PKD; (b) determining a level of \( \alpha-1 \)-microglobulin/bikunin precursor (AMBP) in the sample; and (c) identifying the patient as having PKD if the level is elevated as compared to a control level. In some embodiments of these methods, the PKD is autosomal dominant PKD. In some embodiments of these methods, the PKD is autosomal recessive PKD.

Some embodiments of these methods further include: (d) administering a PKD treatment to the patient. In some embodiments of these methods, the PKD treatment includes a glucosyl ceramide synthase (GCS) inhibitor. In some embodiments of these methods, the GCS inhibitor is selected from the group of: (S)-quinuclidin-3-yl (2-(4′-(2-methoxyethoxy)-[1,1′-biphenyl]-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-(4-((2-methoxy ethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((methoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((methoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; quinuclidin-3-yl (2-(4′-fluoro-[1,1′-biphenyl]-3-yl)propan-2-yl)carbamate; and carbamic acid, N-[1-(2-(4-fluorophenyl)-4-thiazolyl)-1-methylethyl]-, (3S)-1-azabicyclo[2.2.2]oct-3-yl ester. In some embodiments of these methods, the PKD treatment includes a CDK inhibitor (e.g., R-roscovitine or S-CR8).

Some embodiments of these methods further include: (d) imaging one or both kidney(s) in the patient. In some embodiments of these methods, the control level is a threshold level or a level in a healthy subject or a population of healthy subjects.
In some embodiments of these methods, determining the level of AMBP in (b) includes determining the level of AMBP protein. In some embodiments of these methods, (b) includes contacting the sample with an antibody that binds specifically to AMBP protein.

Also provided herein are methods of monitoring a polycystic kidney disease (PKD) patient that include: (a) providing a first sample including a biological fluid obtained from a PKD patient; (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the first sample; (c) providing a second sample including a biological fluid from the patient after step (b) and determining a level of AMBP in the second sample; and (d) identifying the patient as having improving or static PKD if the level in the second sample is not higher than the level in the first sample. In some embodiments of these methods, the PKD patient has autosomal dominant PKD. In some embodiments of these methods, the PKD patient has autosomal recessive PKD. In some embodiments of these methods, the first and second samples include urine.

Some embodiments of these methods further include: (e) administering the same treatment to a patient identified as having improving or static PKD. In some embodiments of these methods, determining the level of AMBP in (b) and (c) include determining the level of AMBP protein. In some embodiments of these methods, (b) and (c) include contacting the sample with an antibody that binds specifically to AMBP protein.

Also provided are kits comprising, consisting, or consisting essentially of: an antibody that specifically binds to α-1-microglobulin/bikunin precursor (AMBP) protein; and an antibody that specifically binds to an additional protein marker of polycystic kidney disease.

As used herein, the word "a" before a noun represents one or more of the particular noun. For example, the phrase "a marker" represents "one or more markers."

The term "patient" means a vertebrate, including any member of the class mammalia, including humans, domestic and farm animals, and zoo, sports or pet animals, such as mouse, rabbit, pig, sheep, goat, cattle, horse (e.g., race horse), and higher primates. In preferred embodiments, the patient is a human.

The term "biological fluid" means any fluid obtained from a mammalian patient (e.g., blood, plasma, serum, or other blood fractions, lymph, urine, cerebrospinal fluid, ascites, saliva, breast milk, tears, vaginal discharge, amniotic fluid, lavage, semen, glandular secretions, exudate, and contents of cysts or feces). In preferred embodiments, the biological fluid is urine, blood, serum, or plasma.
Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is an immunoblot showing the level of AMBP protein in urine from two healthy patients (left three lanes; normal) and the level of AMBP protein in urine from ADPKD patients having total kidney volumes ranging from 256 mL to 1972 mL.

Figure 2 is a graph showing the quantitated level of AMBP protein in urine in both healthy patients (open circles; normal) and patients having ADPKD (dark circles) (quantitated from the immunoblot of Figure 1) as a function of total kidney volume (TKV) in the patients.

Figure 3 is an immunoblot showing the level of AMBP protein in kidney lysates from healthy patients (N; normal) and three patients having ADPKD (P1, P2, and P3).

Figure 4 is an immunofluorescence micrograph of kidney tissue from a healthy (normal patient) (left panel) and a patient having ADPKD (right panel). AMBP protein is shown and localizes to the proximal tubules (PT) (indicated by arrows), lipoprotein lipase (LTL), a marker of PT, and 4',6-diamidino-2-phenylindole (DAPI), a DNA stain, are shown.

Figure 5 is a graph showing the fold-expression level of AMBP mRNA in kidneys from a 64-day-old jck mice as compared to wild type (WT) mice. The expression of AMBP mRNA in kidneys from 64-day-old jck mice and WT mice was determined using RT-PCR.

Figure 6 is an immunoblot showing the level of AMBP protein in kidney lysates from wild type (WT) control mice and jck mice at days 26, 50, and 64 after birth.

Figure 7 is a graph showing the quantitated level of AMBP protein in kidney lysates from wild type (WT) control mice and jck mice at days 26, 50, and 64 after birth (quantitated from the immunoblot of Figure 6).
Figure 8 is an immunofluorescence micrograph of kidney tissue from a wild type control mouse (upper left panel) and a jck mouse at days 26, 50, and 64 after birth (upper right, lower left, and lower right, respectively). AMBP protein, LTL, and DAPI are shown.

Figure 9 is an immunoblot showing the level of AMBP protein in urine collected from a wild type mouse and a jck mouse at days 26, 33, day 41, day 48, and day 64 after birth.

Figure 10 is a graph showing the quantitated level of AMBP protein in urine collected from a wild type mouse and a jck mouse at day 26, day 33, day 41, day 48, and day 64 after birth (quantitated from the immunoblot of Figure 9).

Figure 11 is an immunoblot showing the levels of AMBP protein in kidney lysates from a wild type (control) mouse and a Pkd1 cKO mouse at day 26 and day 64 after birth.

Figure 12 is an immunofluorescence micrograph of kidney tissue of a wild type (control) mouse (left panel), a Pkd1 cKO mouse at 26 days after birth, and a Pkd1 cKO mouse at 64 days after birth. AMBP protein and LTL are shown, "PT" indicates a proximal tube, and "CY" indicates a cyst.

Figure 13 is an immunoblot showing the level of AMBP protein in urine collected from a wild type (control; C) mouse or a Pkd1 cKO mouse at days 26, 33, 41, 48, and 64 after birth.

Figure 14 is a graph showing the quantitated level of AMBP protein in urine collected from control (light data) and three Pkd1 cKO mice at days 26, 33, 41, 48, and 64 after birth (quantitated from the immunoblot of Figure 13).

Figure 15 is a schematic of an animal model study to test the effect of 0.2% roscovitine present in feed on day 26 to day 64 after birth on AMBP protein levels in jck mice.

Figure 16 is a graph showing the kidney to body weight measurements in 64-day-old jck mice which received either vehicle or 0.2% roscovitine in feed on day 26 to day 64 after birth.

Figure 17 is a graph showing the level of AMBP protein in kidney lysate and urine of 64-day-old jck mice which received either vehicle or 0.2% roscovitine in feed on day 26 to day 64 after birth.

Figure 18 is a schematic of an animal model study to test the effect of different treatment schedules of S-CR8 on cystic volume and AMBP levels in jck mice. Jck mice in the study were treated with one of the following S-CR8 treatment schedules (each starting on
day 26 after birth): (A) intraperitoneal injection once a day for 5 weeks; (B) three weeks of daily intraperitoneal injection, and two weeks of no treatment; and (D) one week of daily intraperitoneal injection, and four weeks of no treatment. Urine was collected from all mice at day 64 after birth.

Figure 19 is a graph showing cystic volume (as a percentage of body weight) in jck mice administered a vehicle daily for five weeks (starting at day 26 after birth) and jck mice administered S-CR8 treatment schedules A, B, and D (as described in Figure 18).

Figure 20 is an immunoblot showing the level of AMBP protein in urine collected at day 64 after birth in mice administered a vehicle daily for five weeks (starting at day 26 after birth) and jck mice administered S-CR8 treatment schedules A, B, and D (as described in Figure 18).

Figure 21 is a graph showing the quantitated level of AMBP protein in urine collected at day 64 after birth in mice administered a vehicle daily for five weeks (starting at day 26 after birth) and jck mice administered S-CR8 treatment schedules A, B, and D (as described in Figure 18) (quantitated from the immunoblot of Figure 20).

Figure 22 is an immunoblot showing the level of AMBP protein in urine and kidney lysate obtained from 64-day-old jck mice following chronic treatment between day 26 to day 64 after birth with GCSI.

**DETAILED DESCRIPTION**

Provided herein are methods for determining the efficacy of treatment for PKD (e.g., ADPKD or ARPKD) in a patient, diagnosing PKD in a patient, staging PKD in a patient, and monitoring PKD in a patient that include determining a single or multiple levels of AMBP. Also provided are kits comprising: an antibody that specifically binds to AMBP, and an antibody that specifically binds to an additional protein marker of PKD (e.g., any of the additional protein markers of PKD described herein or known in the art). Non-limiting aspects of these methods are described below. As can be appreciated in the art, the various aspects described below can be used in any combination without limitation.

**Polycystic Kidney Disease**

The methods described herein can further include a step of identifying or diagnosing a patient as having PKD. Non-limiting examples of diagnosing a patient as having PKD are provided herein and are described below.
In other examples, a patient is identified as having PKD based on the observation or assessment of one or more symptoms of the following symptoms in a patient: high blood pressure, back or side pain, headache, increased size of abdomen, presence of blood in urine, frequent urination, kidney stones, kidney failure, urinary tract or kidney infections, cysts on the kidney, cysts on the liver, pancreatic cysts, mitral valve prolapse, aneurysms, nausea, vomiting, left ventricular hypertrophy, hernia, diverticulitis, fatigue, poor appetite, weight loss, trouble concentrating, dry/itchy skin, muscle cramps, swelling in feet and ankles, mild to moderate depression, and bubbly urine. PKD can also be diagnosed in a subject by performing a genetic test (see, e.g., PKD1 genetic diagnostic tests from a variety of vendors including Athena Diagnostics (Worcester, MA) and CGC Genetics (Porto, Portugal); PKD2 genetic diagnostic tests from a variety of vendors including Centogene AG (Germany), PreventionGenetics (Marshfield, WI), GCG Genetics (Portugal), and Invitae Corporation (San Francisco, CA); and PKHD1 genetic diagnostic tests are available from a variety of vendors including Centogene AG (Germany), Prevention Genetics (Marshfield, WI), Counsyl (San Francisco, CA), and Invitae (San Francisco, CA)). The detection of mutations or deletions of the PKD1 and/or PKD2 genes can be used to diagnose ADPKD, and the detection of mutations or deletions in PKHD1 can be used to diagnose ARPKD.

PKD (e.g., ADPKD and ARPKD) can be diagnosed by performing imaging studies. For example, ultrasound, computerized tomography (CT), and magnetic resonance imaging (MRI) can be used to look for cysts on the kidney(s) and to determine the total kidney volume (TKV) or height-adjusted total kidney volume (htTKV). For example, the detection of at least two cysts (e.g., at least three, four, five, or six cysts) on each kidney by age 30 in a patient (e.g., a patient with a family history of the disease) can confirm the diagnosis of PKD. The detection of a multicystic dysplastic kidney(s) in a fetus (e.g., a fetus that is greater than 14 weeks of gestation) can be used to diagnose ARPKD. In addition, the amniotic fluid from a fetus can be used to detect a mutation or deletion in PKHD1 (e.g., using any of the genetic diagnostic tests for PKHD1 described herein or known in the art).

PKD can also be diagnosed or identified in a subject, in part, by determining a patient's kidney function. For example, PKD can be diagnosed and identified in part by measuring one or more of a patient's creatinine level (e.g., a level of creatinine greater than 1.3 mg/dL indicating that the patient has PKD), glomerular filtration rate (e.g., a rate that is below 80 mL/minutes indicates that the patient has PKD), and blood urea nitrogen (e.g., a blood urea nitrogen level of greater than 20 mg/dL).
The PKD patients described herein can be diagnosed or identified using any of the methods described or provided herein, or any methods known in the art. The PKD patient can be in utero (e.g., a fetus with a gestational age greater than 14 weeks, 15 weeks, 17 weeks, 20 weeks, 25 weeks, 30 weeks, or 35 weeks), an infant, an adolescent (between 13 and 18 years old (e.g., between 13 and 15 years old or between 15 and 18 years old)), or an adult (greater than 18 years old (e.g., greater than 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 years old)). The PKD patient can be a female (e.g., a pregnant female) or can be a male. The PKD patient may already be receiving a treatment for PKD. In other examples, the PKD patient may not have received a treatment for a PKD. In additional examples, the PKD patient may have received a previous treatment for PKD and the previous treatment was therapeutically unsuccessful (e.g., lead to the development of negative adverse side effects, did not reduce the rate of development and/or growth of cysts, and/or did not reduce the rate of loss in the function of the patient's kidney(s)). The PKD patient may be a participant in a clinical study.

PKD Treatments

Additional examples of GCS inhibitors are described in WO 14/043068 (incorporated herein by reference). For example, a GCS inhibitor can have a structure represented by Formula I below.

![Chemical structure](image)

wherein:

- n is 1, 2, or 3;
- m is 0 or 1;
- p is 0 or 1;
- t is 0, 1, or 2;
- E is S, O, NH, NOH, NNO2, NCN, NR, NOR or NSO2R;
- X1 is CR1 when m is 1 or N when m is 0;
- X2 is O, -NH, -CH2, SO2, NH-SO2, CH(Ci-Ce) alkyl or -NR2;
- X3 is a direct bond, O, -NH, -CH2-, CO, -CH(Ci-Ce) alkyl, SO2NH, -CO-NH-, or NR3;
- X4 is a direct bond, CR4R5, CH2CR4R5 or CH2-(Ci-Ce) alkyl-CR4R5;
- X5 is a direct bond, O, S, SO2, CR4R5, (Ci-Ce)alkyl, (Ci-Ce)alkyloxy, -0-(Ci-Ce)alkyl, (Ci-Ce)alkenyl, (Ci-Ce)alkenylxy, -R7-(C3-Cio)cycloalkyl, (C3-Cio)cycloalkyl-R7-, -R7-(C6-Cio)aryl, (C6-Cio)aryl-R7-, -R7-(C2-C9)heteroaryl, (C2-C9)heteroaryl-R7-, (C2-C9)heterocycloalkyl, and (C2-C9)heterocycloalkyl-R7-, wherein R7 is a direct bond, O, S, SO2, CR4R5, (Ci-Ce)alkyl, (Ci-Ce)alkyloxy, -O- (Ci-Ce)alkyl, (Ci-Ce)alkenyl, (Ci-C6)alkenylxy; and further wherein when X5 is defined as -R7-(C3-Cio)cycloalkyl, (C3-Cio)cycloalkyl-R7-, -R7-(C6-Cio)aryl, (C6-Cio)aryl-R7-, -R7-(C2-C9)heteroaryl, (C2-C9)heteroaryl-R7-, -R7-(C2-C9) heterocycloalkyl, and (C2-C9)heterocycloalkyl-R7-, wherein the (C3-Cio)cycloalkyl, (C6-C12) aryl, (C2-C9)heteroaryl, (C2-C9) heterocycloalkyl groups are optionally substituted by one or more substituents selected from the group consisting of halo, (Ci-C6)alkyl, (C1-C6) alkyllynyl, amino, (C1-C6) alkylamino, (Ci-C6) dialkylamino, (Ci-
(Ce)alkoxy, 0(C 3-C 6 cycloalkyl), (C3-Ce) cycloalkoxy, nitro, CN, OH, (Ci-Ce)alkyloxy, (C3-
C6) cycloalkyl, (C1-C6) alkoxycarbonyl, (C1-C6) alkyllcarbonyl, (Ci-C6)halo alkyl, (C2-
c9) heterocycloalkyl, R8R9N-CO- wherein R8 and R9 are each independently selected from the
group consisting of hydrogen and (C 1-C 6) alkyl or R8 and R9 can be taken together with
the nitrogen to which they are attached to form a (C2-C9) heterocycloalkyl or (C2-
c9) heterocycloalkyl group optionally substituted by one to three halo groups, (Ci-
c6)alkylsulfonyl optionally substituted by one or two groups selected from (Ci-Ce)alkoxy
and (C3 -Cio)cycloalkyl;

(Ci-Ce) alkyl substituted by one to four substituents selected from the group
consisting of halo, hydroxy, cyano, (Ci-Ce)alkoxy, (Ci-C6)alkoxy(Ci-C6)alkoxy, (C2-
c9) heterocycloalkyl, (C2-C9) heteroaryl optionally substituted by (Ci-Ce)alkoxy; or (C3-
Cio)cycloalkoxy optionally substituted by (Ci-Ce)alkoxy; and

(Ci-Ce)alkyloxy substituted by one to four substituents selected from the group
consisting of halo, hydroxy, cyano, (Ci-Ce)alkoxy, (Ci-C6)alkoxy(Ci-C6)alkoxy, (C2-
c9) heterocycloalkyl, (C2-C9) heteroaryl optionally substituted by (Ci-Ce)alkoxy; or (C3-
Cio)cycloalkoxy optionally substituted by (Ci-Ce)alkoxy;

R is (Ce-Ci2)aryl, (C3-C9) heteroaryl, (Ci-Ce)alkyl, (C2-C9) heteroaryl(Ci-C 6)alkyl;
R1 is H, CN, (Ci-C 6) alkyllcarbonyl, or (Ci-C 6) alkyl;
R2 and R3 are each independently -H, (Ci-C6) alkyl optionally substituted by one or
more substituents selected from the group consisting of halogen, (Ci-Ce) alkyl, (C6-
Ci2)aryl, (C2-C9) heteroaryl, (Ci-C6) alkyll(C6-Ci2)aryl, halo(C6-Ci2)aryl, and halo(C2-
C9) heteroaryl, or optionally when X 2 is -NR 2 and X 3 is -NR 3, R 2 and R 3 may be taken together with the
nitrogen atoms to which they are attached form a non-aromatic heterocyclic ring optionally
substituted by with one or more substituents selected from halogen, (Ci-C6) alkyl, (C6-
Ci2)aryl, (C2-C9) heteroaryl, (Ci-C6) alkyll(C6-Ci2)aryl, halo(C6-Ci2)aryl, and halo(C2-
C9) heteroaryl;

R 4 and R 5 are independently selected from H, (Ci-C6) alkyl, or taken together with the
carbon to which they are attached to form a spiro (C3-Cio)cycloalkyl ring or spiro (C3-
Cio)cycloalkoxy ring;

R 6 is -H, halogen, -CN, (C6-Ci 2) aryl, (C6-Ci 2) arloxy, (Ci-C 6) alkyloxy; (Ci-C 6) alkyl
optionally substituted by one to four halo or (Ci-C6) alkyl;

A 1 is (C2-C6) alkylnyl; (C3-Cio) cycloalkyl, (C6-Ci2)aryl, (C2-C9) heteroaryl, (C2-
c9) heterocycloalkyl or benzo(C2-C9) heterocycloalkyl wherein A 1 is optionally substituted
with one or more substituents selected from the group consisting of halo, (Ci-Ce)alkyl optionally substituted by one to three halo; (Ci-Ce)alkenyl, amino, (Ci-C6)alkylamino, (Ci-C6) dialkylamino, (Ci-C6)alkoxy, nitro, CN, -OH, (Ci-C6)alkyloxy optionally substituted by one to three halo; (Ci-Ce)alkoxy carbonyl, and (Ci-C6) alkylcarbonyl;

$$A^2$$ is H, (C3-Cio)cycloalkyl, (C6-C12)aryl, (C2-C9)heteroaryl, (C2-

C9)heterocycloalkyl or benzo(C2-C9)heterocycloalkyl wherein $$A^2$$ is optionally substituted with one or more substituents selected from the group consisting of halo, (Ci-Ce)alkyl, (Ci-C6)alkylenyl, amino, (Ci-C6) alkylamino, (Ci-C6)dialkylamino, (Ci-Ce)alkoxy, 0(C3-C6 cycloalkyl), (Cs- Ce) cycloalkoxy, nitro, CN, OH, (Ci-C6)alkyloxy, (C3-Ce) cycloalkyl, (C1-

C6)alkyloxy, (C1-C6)alkyloxycarbonyl, (C1-C6) halo alkyl, (C2-C9)heterocycloalkyl,

$$^{\Delta }$$R^\Delta -CO-$$\_{\Delta }$$, wherein $$R^8$$ and $$R^9$$ are each independently selected from the group consisting of hydrogen and (Ci-C6)alkyl or $$R^8$$ and $$R^9$$ can be taken together with the nitrogen to which they are attached to form a (C2-C9)heterocycloalkyl or (C2-C9)heterocycloalkyl group optionally substituted by one to three halo groups, (Ci-C6)alkylsulfonyl optionally substituted by one or two groups selected from (Ci-Ce)alkoxy and (C3-Cio)cycloalkyl;

(Ci-Ce)alkyl substituted by one to four substituents selected from the group consisting of halo, hydroxy, cyano, (Ci-Ce)alkoxy, (Ci-C6)alkoxy(Ci-C6)alkoxy, (C2-C9)heterocycloalkyl, (C2-C9)heteroaryl optionally substituted by (Ci-Ce)alkoxy; or (C3-Cio)cycloalkoxy optionally substituted by (Ci-Ce)alkoxy; and

(Ci-Ce)alkyloxy substituted by one to four substituents selected from the group consisting of halo, hydroxy, cyano, (Ci-Ce)alkoxy, (Ci-C6)alkoxy(Ci-C6)alkoxy, (C2-C9)heterocycloalkyl, (C2-C9)heteroaryl optionally substituted by (Ci-Ce)alkoxy; or (C3-Cio)cycloalkoxy optionally substituted by (Ci-Ce)alkoxy;

with the proviso that the sum of n + t + Y + z is not greater than 6;

with the proviso that when p is 0; X^2 is NH-SO_{2} and X^3 is NH;

with the proviso that when n is 1; t is O; y is 1; z is 1; X^2 is NH; E is O; X^3 is NH;

$$A^2$$ is H and $$X^5$$ is a direct bond; $$A^1$$ is not unsubstituted phenyl, halophenyl or isopropenylphenyl;

with the proviso that when n is 1; t is O; y is 1; z is 1; X^2 is O; E is O; X^3 is NH; $$A^1$$ is (C6-C12)aryl and $$X^5$$ is a direct bond; $$A^2$$ is H and $$R^4$$ is H then $$R^5$$ is not cyclohexyl; and

with the proviso that when n 3 is O, -NH, -CH2-, CO, -CH(Ci-Ce) alkyl, SO2NH, -CO-NH- or -NR_{3}; and $$X^4$$ is CR^4_{R^5}, CH_{2}CR^4_{R^5} or CH_{2}(Ci-Ce) alkyl-CR^4_{R^5}; then $$A^2$$
must be (C3-C10)cycloalkyl, (C6-C12)aryl, (C2-C9)heteroaryl, (C2-C9)heterocycloalkyl or benzo(C2-C9)heterocycloalkyl substituted with one or more substituents selected from the group consisting of (C2-C9)heterocycloalkyl, R^8R^9N-CO- wherein R^8 and R^9 are each independently selected from the group consisting of hydrogen and (Ci-C6)alkyl or R^8 and R^9 can be taken together with the nitrogen to which they are attached to form a (C2-C9)heterocycloalkyl or (C2-C9)heterocycloalkyl group optionally substituted by one to three halo groups, (Ci-C6)alkylsulfonyl optionally substituted by one or two groups selected from (Ci-C6)alkoxy and (C3-C10)cycloalkyl;

(Ci-Ce)alkyl substituted by one to four substituents selected from the group consisting of hydroxy, cyano, (Ci-Ce)alkoxy, (Ci-C6)alkoxy(Ci-C6)alkoxy, (C2-C9)heterocycloalkyl, (C2-C9)heteroaryl optionally substituted by (Ci-Ce)alkoxy; or (C3-C10)cycloalkoxy optionally substituted by (Ci-Ce)alkoxy; or

(Ci-Ce)alkyloxy substituted by one to four substituents selected from the group consisting of hydroxy, cyano, (Ci-Ce)alkoxy, (Ci-C6)alkoxy(Ci-C6)alkoxy, (C2-C9)heterocycloalkyl, (C2-C9)heteroaryl optionally substituted by (Ci-Ce)alkoxy; or (C3-C10)cycloalkoxy optionally substituted by (Ci-Ce)alkoxy.

Additional exemplary GCS inhibitors include:

1-azabicyclo[2.2.2]oct-3-yl [2-(2,4'-difluorobiphenyl-4-yl)propan-2-yl]carbamate;

1-azabicyclo[2.2.2]oct-3-yl [2-[4-(1,3-benzothiazol-6-yl)phenyl]propan-2-yl] carbamate;

1-azabicyclo[3.2.2]non-4-yl [1-[5 -(4-fluorophenyl)pyridin -2-yl] cyclopropyl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [1-[3-(4-fluorophenoxy)phenyl]cyclopropyl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [1-[4-(3-benzothiazol-5-yl)phenyl]cyclopropyl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [l-(4'-fluoro-3 '-methoxybiphenyl-4yl)cyclopropyl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [3-(4'-fluorobiphenyl-4-yl)oxetan-3-yl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [1-[6-(4-fluorophenoxy)pyridin-2-yl]cyclopropyl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [3-(4'-fluorobiphenyl-4-yl)pentan-3-yl ] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [2-[2-(4-fluorophenyl)-2H -indazol-6-yl]propan-2 yl] carbamate;

1-azabicyclo[2.2.2]oct-3-yl [2-[2-(IH-pyrrol-1-yl)pyridin-4-yl]propan-2-yl] carbamate;
1-(3-ethyl-l-azabicyclo[2.2.2]oct-3-yl)-3-[1-(4'-fluorobiphenyl-4-yl)cyclopropyl]urea;
N-(l-azabicyclo[2.2.2]oct-3-yl)-N'-[l-(4'-fluorobiphenyl-4-yl)cyclopropyl] ethanedi amide;
1-azabicyclo[2.2.2]oct-3-yl (1-[(4,4difluorocyclohexyl)oxy Jphenyl] cyclopropyl) carbamate;
1-(4-methyl-l-azabicyclo[3.2.2]non-4-yl)-N-[l-(5-phenylpyridin-2-yl)cyclopropyl]-l-methyl-3-(3-methyl-l-azabicyclo[2.2.2]oct-3-yl)urea;
1-[l-(4'-fluorobiphenyl-4-yl)cyclopropyl]-l-methyl-3-(3-methyl-l-azabicyclo[2.2.2]oct-3-yl)urea;
1-[l-(4'-fluorobiphenyl-4-yl)propan-2-yl]-3-(3-methyl-l-azabicyclo[2.2.2]oct-3-yl)urea;
2-(1-azabicyclo [3.2.2 ]non -4-yl)-N -[ l-(5 -phenylpyridin -2-yl )cyclopropyl] acetamide;
3-(4'-fluorobiphenyl-4-yl)-3-methyl-N-(4-methyl-l-azabicyclo[3.2.2]non-4-5 yl)butanamide;
N-[2-(biphenyl-4-yl)propan-2-yl]-N’-(3-methyl-l-azabicyclo[2.2.2]oct-3-yl)sulfuric diam ide;
N-[2-(4'-fluorobiphenyl-4-yl)propan-2-yl]-N’-(3-methyl-l-azabicyclo[2.2.2]oct-3-yl)sulfuric diam ide;
1-(3-butyl-l-azabicyclo[2.2.2]oct-3-yl)-3-[2-[l-(4-fluorophenyl)-IH-pyrazol-4-yl]propan-2-yl] urea;
1-azabicyclo[2.2.2]oct-3-yl [4-(4-fluorophenyl)-2-methylbut-3-yn-2-yl] carbamate;
1-(3-butyl-l-azabicyclo[2.2.2]oct-3-yl)-3-[4-(4-fluorophenyl)-2-methylbut-3-yn-2-yl]urea;
N-[l-(4'-fluorobiphenyl-4-yl)cyclopropyl]-l ,4-diazabicyclo[3 .2.2]nonan-4-carboxamide;
1-(2-(4'-fluoro-[l, 1'-biphenyl]-4-yl)propan-2-yl)-3-(3-methyl-l-azabicyclo[3.2.2]nonan-3-yl)urea;
1-(2-(4'-fluoro-[l, 1'-biphenyl]-4-yl)propan-2-yl)-3-(4-methyl-l-azabicyclo[4.2.2]decan-4-yl)urea;
1-((2-(4′-fluoro-[1, 1′-biphenyl]-4-yl)propan-2-yl)-3-(3-methyl-1-azabicyclo[4.2.2]decan-3-yl)urea; and
1-((2-(4′-fluoro-[1, 1′-biphenyl]-4-yl)propan-2-yl)-3-(5-methyl-1-azabicyclo[4.2.2]decan-5-yl)urea.

Additional examples of GCS inhibitors are listed below.

((S)-Quinuclidin-3-yl (2-(4′-(2-methoxyethoxy)-[1,1′-biphenyl]-4-yl)propan-2-yl)carbamate)

(4-Fluoro-1-(5-fluoro-4-(2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide)
(4-Fluoro-L-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide)

(4-Fluoro-L-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-L-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide)

(4-Fluoro-L-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3 methylquinuclidin-3-yl)piperidine-4-carboxamide)
(4-Fluoro-l-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide)

(4-Fluoro-l-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide)

(4-Fluoro-l-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide)
Another exemplary GCS inhibitor is: carbamic acid, N-[1-(2-(4-fluorophenyl)-4-thiazolyl)-1-methylethyl]-, (3S)-l-azabicyclo[2.2.2]oct-3-yl ester as represented by Formula II below:
Another exemplary GCS inhibitor is: quinuclidin-3-yl (2-(4'-fluoro-[1,1'-biphenyl]-3-yl)propan-2-yl)carbamate as represented by Formula III below:


Another example of a treatment for PKD is hemodialysis or peritoneal dialysis. A further example of a treatment for PKD is the surgical transplantation of a kidney.

**Determination of a Level of a Marker(s)**

The methods provided herein include the determination of the level(s) of a marker(s) (e.g., AMBP and/or AMBP and at least one additional marker of PKD), in at least one sample from a patient (e.g., a PKD patient). For example, the level(s) of a marker(s) (e.g., AMBP and/or AMBP and at least one additional marker of PKD) can be determined in a sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) from the patient (e.g., a PKD patient). In some examples, the marker(s) is a protein. In other examples, the marker(s) is an mRNA encoding the marker protein.
Methods for determining the levels of the marker(s) described herein (e.g., AMBP or AMBP and one or more additional markers of PKD) are well understood in the art. For example, the protein level(s) of a marker(s) described herein (e.g., AMBP or AMBP and one or more additional markers of PKD) can be determined using an antibody-based assay (e.g., an enzyme-linked immunosorbent assay, antibody array, antibody-labeled beads, or immunoblots). Exemplary antibodies that can be used in these antibody-based assays are described in the Examples. Additional antibodies that can be used in the antibody-based assays are known in the art. Non-limiting examples of antibodies that specifically bind to human AMBP are commercially available from Santa Cruz Biotechnology (Dallas, TX), Thermo Fisher Scientific (Waltham, MA), and Altas Antibodies (Stockholm, Sweden). Non-limiting examples of antibodies that specifically bind to PCNA are commercially available from Santa Cruz Biotechnology (Dallas, TX), Abeam (Cambridge, MA), and Acris Antibodies (San Diego, CA). Non-limiting examples of antibodies that specifically bind to cyclin D1 are commercially available from Santa Cruz Biotechnology (Dallas, TX), Cell Signaling Technology (Danvers, MA), and Abeam (Cambridge, MA). Non-limiting examples of antibodies that specifically bind to cyclin D3 are commercially available from Santa Cruz Biotechnology (Dallas, TX), Abeam (Cambridge, MA), and Novus Biologicals (Littleton, CO). Non-limiting examples of antibodies that specifically bind to MEK are commercially available from Santa Cruz Biotechnology (Dallas, TX), Sigma-Aldrich (St. Louis, MO), and Cell Signaling Technology (Danvers, MA). Non-limiting examples of antibodies that specifically bind to S6 are commercially available from Santa Cruz Biotechnology (Dallas, TX), Thermo Fisher Scientific (Waltham, MA), and GenWay Biotech. Inc. (San Diego, CA). Non-limiting examples of antibodies that specifically bind to pS6 are commercially available from Cell Signaling Technology (Danvers, MA), Abeam (Cambridge, MA), and Abbiotec (San Diego, CA). Non-limiting examples of antibodies that specifically bind to ERK are commercially available from Cell Signaling Technology (Danvers, MA), Santa Cruz Biotechnology (Dallas, TX), and Thermo Fisher Scientific (Waltham, MA). Non-limiting examples of antibodies that specifically bind to pERK are commercially available from Cell Signaling Technologies (Danvers, MA), EMD Millipore (Billerica, MA), and eBioscience (San Diego, CA). Non-limiting examples of antibodies that specifically bind to AKT are commercially available from Cell Signaling Technologies (Danvers, MA), Abeam (Cambridge, MA), and Santa Cruz Biotechnology (Dallas, TX). Non-limiting examples of antibodies that specifically bind to pAkt are commercially
available from Cell Signaling Technologies (Dallas, TX), EMD Millipore (Billerica, MA),
and Signalway Antibody (College Park, MD). Non-limiting examples of antibodies that
specifically bind to caspase-2 are commercially available from Santa Cruz Biotechnology
(Dallas, TX), Acris Antibodies (San Diego, CA), and Abeam (Cambridge, MA). Anon-
limiting example of an antibody that specifically binds to RBBP is commercially available
from Santa Cruz Biotechnology (Dallas, TX).

Methods of making antibodies that specifically bind to a marker (e.g., AMBP or any
of the additional markers of PKD described herein) are also well known in the art.
Additional methods for determining the protein level(s) of the marker(s) include mass
spectrometry, liquid chromatography (e.g., high performance liquid chromatography) mass
spectrometry (LC-MS), and liquid chromatography (e.g., high performance liquid
chromatography) tandem mass spectrometry (LC-MS/MS). Non-limiting examples of
methods for determining a protein level of a marker in a sample including a biological fluid
are described in Písitkun et al. (Proteomics Clin. Appl. 6:268-278, 2012). These exemplary
methods of determining the level(s) of the marker(s) (e.g., AMBP or AMBP and at least one
additional marker of PKD) can be used in any of the methods provided herein.

The mRNA level(s) of the marker(s) described herein (e.g., AMBP or AMBP and at
least one additional marker of PKD) can be determined, e.g., using a polymerase chain
reaction (PCR)-based assay (e.g., real-time PCR and reverse-transcriptase PCR). Additional
methods for determining the mRNA level of each marker include the use of a gene chip.
Further examples of methods for determining the mRNA level of a marker in a sample
including a biological fluid are described in Chen et al. (Lab Chip 10:505-511, 2010),
Schageman et al. (BioMedRes. Int., Article ID 253957, 2013), and Alvarez et al. (Kidney
Inter. 82:1024-1032, 2012). Additional methods for determining an mRNA level of a marker
are well known in the art.

In some examples, a sample (e.g., a sample comprising a biological fluid or kidney
tissue, such as a kidney biopsy sample) from a subject can be stored for a period of time (e.g.,
stored at least 1 hour (e.g., at least 2, 4, 6, 8, 12, or 24 hours, or at least 1, 2, 3, 4, 5, 6, 7, 14,
or 21 days, e.g., at a temperature of about 10 °C, about 0 °C, about -20 °C, about -40°C,
about -70 °C, or about -80 °C) before the level(s) of the marker(s) are determined in the
sample. Some examples further include a step of concentrating a sample including a
biological fluid before the level(s) of the marker(s) (e.g., AMBP or AMBP and at least one
additional marker of PKD) is determined.
The level(s) of AMBP (and optionally the level(s) at least one additional marker of PKD) can be determined in a sample (e.g., a sample including a biological fluid or kidney tissue (e.g., a kidney biopsy sample) in any of the methods described herein. A description of AMBP and exemplary additional markers of PKD is provided below.

**AMBP**

Alpha-1- microglobulin/bikunin precursor (AMBP) is a 39 kDa pre-pro-protein having 352 amino acids. The amino acid sequence of human AMBP is shown in SEQ ID NO: 1. AMBP is typically digested intracellularly to first remove a signal sequence (the first 19 amino acids of SEQ ID NO: 1) to yield a pro-protein (illustrated herein as SEQ ID NO: 2), and then is further cleaved by proteases to yield three different downstream protein products: alpha-1-microglobulin (having an amino acid sequence shown in SEQ ID NO: 3), bikunin (having an amino acid sequence shown in SEQ ID NO: 4), and trypstatin (having an amino acid sequence shown in SEQ ID NO: 5). Human AMBP (i.e., SEQ ID NO: 1) may be glycosylated at one or more of the following amino acid positions in SEQ ID NO: 1: the threonine at amino acid position 24, the asparagine at amino acid position 36, the asparagine at amino acid position 115, the serine at amino acid position 215, and the asparagine at amino acid position 250. When measuring a protein level of AMBP, the level measured can include the non-glycosylated form of AMBP and one or more forms of AMBP that has been glycosylated at one or more amino acid positions.

**Additional Markers of PKD**

In any of the methods described herein, a level or multiple level(s) of at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) additional marker of PKD can be determined (e.g., in any combination such as those described in U.S. Provisional Patent Application Serial No. 62/033,031). Non-limiting examples of additional markers of PKD are described below.

**PCNA**

Proliferating Cell Nuclear Antigen (PCNA) is a 28.8 kDa protein having 261 amino acids. The amino acid sequence of human PCNA is shown in SEQ ID NO: 6. When measuring a protein level of PCNA, the level measured can include the forms of PCNA that are non-phosphorylated and phosphorylated at the tyrosine at amino acid position 248 in
SEQ ID NO: 6, include only the form of PCNA phosphorylated at the tyrosine at amino acid position 248 in SEQ ID NO: 6, or include only the unphosphorylated form of PCNA.

**Cyclin D1**

Cyclin D1 is a 33.7 kDa protein having 295 amino acids. The amino acid sequence of human cyclin D1 is shown in SEQ ID NO: 7. When measuring a protein level of cyclin D1, the level measured can include the forms of cyclin D1 that are non-phosphorylated and phosphorylated at the threonine at amino acid position 286 in SEQ ID NO: 7, include only the form of cyclin D1 phosphorylated at the threonine at amino acid position 286 in SEQ ID NO: 7, or include only the unphosphorylated form of cyclin D1.

**Cyclin D3**

Cyclin D3 is a protein having 292 amino acids. The amino acid sequence of human cyclin D3 is shown in SEQ ID NO: 8. When measuring a protein level of cyclin D3, the level measured can include the forms of cyclin D3 that are non-phosphorylated and phosphorylated at the serine at amino acid position 279 in SEQ ID NO: 8, include only the form of cyclin D3 phosphorylated at the serine at amino acid position 279 in SEQ ID NO: 8, or include only the unphosphorylated form of cyclin D3.

**MEK**

MAPK-ERK kinase 1 (MEK) is a protein having two isoforms. The first isoform of human MEK has a sequence of 393 amino acids (as shown in SEQ ID NO: 9) and the second isoform of human MEK has a sequence of 367 amino acids (as shown in SEQ ID NO: 10). When measuring the protein level of MEK, the level can include one or more of: the unphosphorylated form of the first isoform of MEK; the unphosphorylated form of the second isoform of MEK; one or more form(s) of the first isoform of MEK including one or more of a phosphorylation at the serine at amino acid position 218 in SEQ ID NO: 9, a phosphorylation at the serine at amino acid position 222 in SEQ ID NO: 9, a phosphorylation at the threonine at amino acid position 286 in SEQ ID NO: 9, a phosphorylation at the threonine at amino acid position 292 in SEQ ID NO: 9, and a phosphorylation at the serine at amino acid position 298 in SEQ ID NO: 9; and one or more form(s) of the second isoform of MEK including one or more of a phosphorylation at the serine at amino acid position 192 of SEQ ID NO: 10, a phosphorylation at the serine at amino acid position 196 of SEQ ID NO:
10, a phosphorylation at the threonine at amino acid position 260 in SEQ ID NO: 10, a phosphorylation at the threonine at amino acid position 266 in SEQ ID NO: 10, and a phosphorylation at the serine at amino acid position 272 in SEQ ID NO: 10.

**S6, pS6, and total S6**

Ribosomal protein S6 (S6) is a 28.7 kDa protein having 249 amino acids. The amino acid sequence of human S6 is shown in SEQ ID NO: 11. The phrase "level of S6," "total S6," or "level of ribosomal protein S6," when referring to a protein level can include the sum of the levels of all detectable forms (e.g., all phosphorylated forms and the unphosphorylated form) of S6. The phosphorylated forms of S6 protein can include one or more of: a phosphorylation of the serine at amino acid position 235 in SEQ ID NO: 11, a phosphorylation of the serine at amino acid position 236 in SEQ ID NO: 11, a phosphorylation of the serine at amino acid position 240 in SEQ ID NO: 11, a phosphorylation at the serine at amino acid position 242 in SEQ ID NO: 11, a phosphorylation at serine at amino acid position 244 in SEQ ID NO: 11, and a phosphorylation at the serine at amino acid position 247 in SEQ ID NO: 11. In some embodiments, the level of S6 can be determined with an antibody that binds to an antigen common to all detectable forms (e.g., all phosphorylated forms and the unphosphorylated form) of S6.

The phrase "level of pS6" or "level of ribosomal protein pS6," when referring to a protein level, means the level (or sum of two or more of the levels) of one or more of a phosphorylated form of ribosomal S6 protein having a phosphorylation at serine at amino acid position 235 in SEQ ID NO: 11, a phosphorylation at serine at amino acid position 236 in SEQ ID NO: 11, or a phosphorylation in the serines at amino acid positions 235 and 236 in SEQ ID NO: 11. The level of pS6 can be determined, e.g., using an antibody or antibodies that specifically bind to an epitope in S6 that includes the phosphorylated serine at amino acid position 235 in SEQ ID NO: 11 and/or the phosphorylated serine at amino acid position 236 in SEQ ID NO: 11.

**ERK**

The phrase "level of ERK," when referring to a protein level, can include the sum of the levels of all detectable forms of ERK1 (e.g., all phosphorylated forms and unphosphorylated forms of each isoform of ERK1) and/or all detectable forms of ERK2 (e.g.,
all phosphorylated forms and unphorylated forms). The first isoform of human ERK1 has a
sequence of 379 amino acids (as shown in SEQ ID NO: 12). The second isoform of human
ERK1 has a sequence of 335 amino acids (as shown in SEQ ID NO: 13). The third isoform
of human ERK1 has a sequence of 357 amino acids (as shown in SEQ ID NO: 14). The first
isoform of human ERK2 has a sequence of 360 amino acids (as shown in SEQ ID NO: 15).
The second isoform of human ERK2 has a sequence of 316 amino acids (as shown in SEQ
ID NO: 16).

The phosphorylated forms of the first isoform of ERK1 can include one or more of: a
phosphorylation of the serine at amino acid position 170 in SEQ ID NO: 12, a
phosphorylation of the threonine at amino acid position 198 in SEQ ID NO: 12, a
phosphorylation of the threonine at amino acid position 202 in SEQ ID NO: 12, a
phosphorylation of the tyrosine at amino acid position 204 in SEQ ID NO: 12, and a
phosphorylation of the threonine at amino acid position 207 in SEQ ID NO: 12. The
phosphorylated forms of the second isoform of ERK1 can include one or more of:
phosphorylation of the serine at amino acid position 170 in SEQ ID NO: 13, a
phosphorylation of the threonine at amino acid position 198 in SEQ ID NO: 13, a
phosphorylation of the threonine at amino acid position 202 in SEQ ID NO: 13, a
phosphorylation of the tyrosine at amino acid position 204 in SEQ ID NO: 13, and a
phosphorylation of the threonine at amino acid position 207 in SEQ ID NO: 13. The
phosphorylated forms of the third isoform of ERK1 can include one or more of: a
phosphorylation of the serine at amino acid position 170 in SEQ ID NO: 14, a
phosphorylation of the threonine at amino acid position 198 in SEQ ID NO: 14, a
phosphorylation of the threonine at amino acid position 202 in SEQ ID NO: 14, a
phosphorylation of the tyrosine at amino acid position 204 in SEQ ID NO: 14, and a
phosphorylation of the threonine at amino acid position 207 in SEQ ID NO: 14. All
detectable forms of ERK1 can be identified, e.g., with an antibody that specifically binds to
an epitope shared between the unphosphorylated forms of the first, second, and third
isoforms of ERK1 and all of the various phosphorylated forms of the first, second, and third
isoforms of ERK1.

The phosphorylated forms of the first isoform of ERK2 can include one or more of: a
phosphorylation at the serine at amino acid position 29 in SEQ ID NO: 15, a phosphorylation
of the threonine at amino acid position 185 in SEQ ID NO: 15, a phosphorylation of the
tyrosine at amino acid position 187 in SEQ ID NO: 15, a phosphorylation of the threonine at
the amino acid position 190 in SEQ ID NO: 15, a phosphorylation of the serine at the amino acid position 246 in SEQ ID NO: 15, a phosphorylation of the serine at amino acid position 248, and a phosphorylation of the serine at amino acid position 284 in SEQ ID NO: 15. The phosphorylated forms of the second isoform of ERK2 can include one or more of: a phosphorylation at the serine at amino acid position 29 in SEQ ID NO: 16, a phosphorylation of the threonine at amino acid position 185 in SEQ ID NO: 16, a phosphorylation of the tyrosine at amino acid position 187 in EQ ID NO: 16, and a phosphorylation of the threonine at the amino acid position 190 in SEQ ID NO: 16. All detectable forms of ERK2 can be identified, e.g., using an antibody that specifically binds to an epitope shared between the unphosphorylated forms of the first and second isoforms of ERK2 and all of the various phosphorylated forms of the first and second isoforms of ERK2.

pERK

The phrase "level of pERK," when referring to a protein level can include the level (or sum of two or more of the levels) of one or more of: a form of the first isoform of ERK1 having a phosphorylation at the threonine at amino acid position 202 in SEQ ID NO: 12, a form of the first isoform of ERK1 having a phosphorylation at the tyrosine at amino acid position 204 in SEQ ID NO: 12, a first isoform of ERK1 having a phosphorylation at threonine at amino acid position 202 and tyrosine at amino acid position 204 in SEQ ID NO: 12, a form of the second isoform of ERK1 having a phosphorylation at the threonine at amino acid position 202 in SEQ ID NO: 13, a form of the second isoform of ERK1 having a phosphorylation at the tyrosine at amino acid position 204 in SEQ ID NO: 13, a form of the second isoform of ERK1 having a phosphorylation at the threonine at amino acid position 202 and the tyrosine at amino acid position 204 of SEQ ID NO: 13, a form of the third isoform of ERK1 having a phosphorylation at the threonine at amino acid position 202 in SEQ ID NO: 14, a form of the third isoform of ERK1 having a phosphorylation at the tyrosine at amino acid position 204 in SEQ ID NO: 14, a form of the third isoform of ERK1 having a phosphorylation at the threonine at amino acid position 202 and the tyrosine at amino acid position 204 in SEQ ID NO: 14, a form of the first isoform of ERK2 having a phosphorylation at the threonine at amino acid position 185 in SEQ ID NO: 15, a form of the first isoform of ERK2 having a phosphorylation at the tyrosine at amino acid position 187 of SEQ ID NO: 15, a form of the first isoform of ERK2 having a phosphorylation at the threonine at amino acid position 185 and the tyrosine at amino acid position 187 of SEQ ID
NO: 15, a form of the second isoform of ERK2 having a phosphorylation at the threonine at amino acid position 185 in SEQ ID NO: 16, a form of the second isoform of ERK2 having a phosphorylation at the tyrosine at amino acid position 187 in SEQ ID NO: 16, and a form of the second isoform of ERK2 having a phosphorylation at the threonine at amino acid position 185 and the tyrosine at amino acid position 187 in SEQ ID NO: 16. The level of pERK can be determined, e.g., using an antibody that specifically binds to an epitope in the first, second, or third isoforms of ERK1 that includes the phosphorylated threonine at amino acid position 202 in SEQ ID NO: 12, 13, or 14 and/or the phosphorylated tyrosine at amino acid position 204 in SEQ ID NO: 12, 13, or 14, respectively, or an antibody that specifically binds to an epitope on the first or second isoforms of ERK2 that includes the phosphorylated threonine at amino acid position 185 in SEQ ID NO: 15 or 16 and/or the phosphorylated tyrosine at amino acid position 187 in SEQ ID NO: 15 or 16, respectively.

**Akt**

Akt is a 55.7 kDa protein having 480 amino acids (as shown in SEQ ID NO: 17). When measuring the protein level of Akt, the level can include two or more of: the unphosphorylated form of Akt and one or more form(s) of Akt including one or more of a phosphorylation at the serine at amino acid position 124 in SEQ ID NO: 17, a phosphorylation at the serine at amino acid position 126 in SEQ ID NO: 17, a phosphorylation at the serine at amino acid position 129 in SEQ ID NO: 12, a phosphorylation at the tyrosine at amino acid position 176 in SEQ ID NO: 17, a phosphorylation at the threonine at amino acid position 308 in SEQ ID NO: 17, a phosphorylation at the threonine at amino acid position 450 in SEQ ID NO: 17, a phosphorylation at the serine at amino acid position 473 in SEQ ID NO: 17, and a phosphorylation at the tyrosine at amino acid position 474 in SEQ ID NO: 17.

**pAkt**

The phrase "level of pAkt," when referring to a protein level can include the level (or sum of two or more of the levels) of one or more a form Akt having a phosphorylation at the serine at amino acid position 473 in SEQ ID NO: 17. The level of pAkt can be determined, e.g., by using an antibody that specifically binds to an epitope in Akt that includes the phosphorylated serine at amino acid position 473 in SEQ ID NO: 17.
Caspase-2
There are three different isoforms of caspase-2 in humans. The first isoform of caspase-2 in its unprocessed form has a total of 452 amino acids (as shown in SEQ ID NO: 18). After processing, the first isoform of caspase-2 forms three subunit peptides: amino acids 170-325 of SEQ ID NO: 18 (caspase-2 subunit p18), amino acids 334-452 of SEQ ID NO: 18 (caspase-2 subunit p13), and amino acids 348-452 of SEQ ID NO: 18 (caspase-2 subunit p12). Amino acids 2-169 of SEQ ID NO: 18 represent the prosequence of the unprocessed form of caspase-2. The second isoform has a total of 313 amino acids (SEQ ID NO: 19). The third isoform has a total of 91 amino acids (SEQ ID NO: 20). A phosphorylated form of the first isoform of caspase-2 has a phosphorylation at the serine at amino acid position 340 in SEQ ID NO: 18. When measuring the protein level of caspase-2, the level can include one or more of the unprocessed form of the first isoform of caspase-2, the caspase-2 subunit p18, the caspase-2 subunit p13, the caspase-2 subunit p12, the form of the first isoform of caspase-2 having a phosphorylation at the serine at amino acid position 340 in SEQ ID NO: 18, and a form of the caspase-2 subunit p13 having a phosphorylation at the serine at amino acid position 7 in caspase-2 subunit p13.

RBBP
Human retinoblastoma binding protein (RBBP) has 425 amino acids (as shown in SEQ ID NO: 21). A phosphorylated form of RBBP has a phosphorylation at the serine at amino acid position 110 in SEQ ID NO: 21. When measuring the protein level of RBBP, the level can include one or both of: the unphosphorylated form of RBBP and a form of RBBP having a phosphorylation at the serine at amino acid position 110 in SEQ ID NO: 21.

Methods of Determining the Efficacy of a Treatment of PKD
Provided herein are methods of determining the efficacy of a treatment for PKD (e.g., ADPKD or ARPKD) in a PKD patient. In some examples, these methods include: (a) providing a first sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) obtained from a PKD patient; (b) determining a level of AMBP in the first sample; (c) administering a treatment for PKD to the PKD patient; (d) providing a second sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) from the PKD patient after step (c) and determining a level of AMBP in the second sample; and (e) identifying the administered treatment as being effective if the level in the
second sample is lower than the level in the first sample. In some examples, these methods include (a) providing a first sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) obtained from a PKD patient; (b) determining a level of AMBP and a level(s) of at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) additional marker of PKD (e.g., PCNA, cyclin D1, cyclin D3, MEK, S6, pS6, ERK, pERK, Akt, pAkt, caspase-2, total S6, and RBBP) in the first sample; (c) administering a treatment for PKD to the PKD patient; (d) providing a second sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) from the PKD patient after step (c) and determining a level of AMBP and the level(s) of the at least one additional marker of PKD in the second sample; and (e) identifying the administered treatment as being effective if (i) the AMBP level in the second sample is lower than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) of the at least one additional marker of PKD in the second sample is lower than the level(s) of the at least one additional marker of PKD in the first sample.

Some embodiments further include after (e): (f) administering additional doses of the administered GCS inhibitor identified as being effective (e.g., (S)-quinoclidin-3-yl (2-(4′-(2-methoxyethoxy)-[1, 1′-biphenyl]-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1 azabicyclo[3.2.2] nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl) pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1 azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxy methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4 (methoxy methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1 azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1 azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluorophenyl)-4-thiazolyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1 azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; carbamic acid, N-[1-(2-(4-fluorophenyl)-4-thiazolyl)-1-methylethyl]-, (3S)-1-azabicyclo[2.2.2]oct-3-yl ester (as represented by Formula II); or quinuclidin-3-yl (2-(4′-fluoro-[1, 1′-biphenyl]-3-yl)propan-2-
yl)carbamate (as represented by Formula III)) to the PKD patient. Some examples of the methods further include after (e): (f) administering additional doses of the administered treatment identified as being effective (e.g., a CDK inhibitor, such as S-CR8) to the PKD patient.

In some embodiments, the steps (b) and (d) include determining the level of one (or the levels of two, three, four, five, or six) additional marker(s) of PKD selected from the group of: PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the administered treatment is identified as being effective if (i) the AMBP level in the second sample is lower than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, or six) of the additional marker(s) of PKD in the second sample is less than the level(s) of the at least one additional marker of PKD in the first sample.

In some examples, the determining the levels of AMBP and optionally, the determining of the level of at least one additional biomarker of PKD in (b) and (d) includes determining the protein levels of AMBP and optionally, the protein level(s) of at least one additional marker of PKD. For example, the determining in (b) and (d) can include contacting the samples with antibodies that bind specifically to AMBP protein, and optionally antibodies that bind specifically to the at least one additional marker of PKD. In some embodiments, (b) and (d) include determining the protein level of the additional marker(s) of PKD of one or both of cyclin D1 and MEK.

In some embodiments of any of the methods, the administered treatment in (c) is administration of a glucosyl ceramide synthase synthase (GCS) inhibitor (e.g., any of the GCS inhibitors described herein or known in the art). For example, the GCS inhibitor is selected from the group of: (S)-quinuclidin-3-yl (2-(4’-(2-methoxyethoxy)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-(4-((2methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-((2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-methoxyethoxy)}
phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; carbamic acid, N-[l-[2-(4-fluorophenyl)-4-thiazolyl]-l-methylethyl]-, (3S)-l-azabicyclo[2.2.2]oct-3-yl ester (as represented by Formula II); and quinuclidin-3-yl \((2'-3'-fluoro-1, r-biphenyl)-3-yl)propan-2-yl)carbamate (as represented by Formula III).

In some embodiments of any of the methods, the administered treatment in (c) is administration of a CDK inhibitor (e.g., any of the CDK inhibitors described herein or known in the art, such as R-roscovitine) to the PKD patient.

Some embodiments of any of the methods further include a step of selecting a patient having PKD or diagnosing a patient having PKD (e.g., using any of the exemplary methods of diagnosing PKD described herein). The patient in any of these methods can be any of the patients described herein. For example, a patient having PKD can have previously been administered a treatment for PKD and the treatment was unsuccessful. Some embodiments of any of the methods further include obtaining the first and/or second samples from the PKD patient.

Some embodiments further include recording the identified efficacy of the administered treatment in the patient's medical record (e.g., a computer readable medium).

Some examples further include informing the patient, the patient's family, and/or the patient's primary care physician or attending physician of the identified efficacy of the administered treatment. Some embodiments further include authorizing a refill of an administered treatment identified as being effective.

The difference in time between when the first sample is obtained from the PKD patient and when the second sample is obtained from the PKD subject can be, e.g., between 1 week and 40 weeks, between 1 week and 30 weeks, between 1 week and 20 weeks, between 1 week and 12 weeks, between 1 week and 8 weeks, between 1 week and 4 weeks, between 1 week and 2 weeks, between 2 weeks and 12 weeks, between 2 weeks and 8 weeks, or between 2 weeks and 4 weeks.

**Methods of Diagnosing PKD**

Also provided are methods of diagnosing PKD (e.g., ADPKD or ARPKD) in a patient that include (a) providing a sample including a biological fluid (e.g., urine) or kidney tissue
(e.g., kidney biopsy sample) from a patient suspected of having PKD; (b) determining a level of AMBP in the sample; and (c) identifying the patient as having PKD if the level is elevated, e.g., where the AMBP level exceeds a control level (e.g., a pre-determined threshold level).

In some examples, the methods of diagnosing PKD (e.g., ADPKD or ARPKD) in a patient include (a) providing a sample including a biological fluid (e.g., urine) or kidney tissue (e.g., kidney biopsy tissue) from a patient suspected of having PKD; (b) determining a level of AMP and determining a level(s) of at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) additional marker of PKD (e.g., PCNA, cyclin D1, cyclin D3, MEK, S6, pS6, ERK, pERK, Akt, pAkt, caspase-2, total S6, and RBBP) in the sample; and (c) identifying the patient as having PKD if (i) the level of AMDA is elevated, e.g., as compared to a control level, and (ii) the level of one (or the levels of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) of the at least one additional markers of PKD is elevated as compared to a control level(s).

Some examples of these methods further include after (c): (d) administering a treatment for PKD (e.g., any of the exemplary treatments for PKD described herein) to a patient identified as having PKD. Some embodiments further include after (c): (d) administering a GCS inhibitor (e.g., (S)-quinuclidin-3-yl 2-(4’-(2-methoxyethoxy)-l’-biphenyl)-4-yl)propan-2-yl)carbamate; 4-fluoro-l-(5-fluoro-4-(4-(2methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l -azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4’-(2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-l-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-l-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; carboxamic acid, N-[I-[2-(4-fluorophenyl)-4-thiazolyl]-l -methylthyl]-, (3S)-l-azabicyclo[2.2.2]oct-3-yl ester (as represented by Formula II); or quinuclidin-3-yl 2-(4’-fluoro-l, 1’-biphenyl]-3-yl)propan-2-
yl)carbamate (as represented by Formula III)) to a patient identified as having PKD. Some embodiments further include after (c): (d) administering a CDK inhibitor (e.g., roscovitine) to a patient identified as having PKD. Some embodiments further include after (c): (d) performing one or more additional tests to confirm PKD in the patient (e.g., performing imaging one or both kidney(s) in a patient identified as having PKD).

In some embodiments, the step (b) includes determining the level of one (or levels of two, three, four, five, six, or seven) of additional markers of PKD selected from the group of: PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the patient is identified as having PKD if the subject has a level of AMBP that is elevated as compared to a control level, and the level of one (or the levels of two, three, four, five, or six) of the additional marker(s) of PKD is elevated as compared to a control level.

In some examples, the determining the levels of AMBP and optionally, the determining of the level of at least one additional biomarker of PKD in (b) includes determining the protein level of AMBP and optionally, the protein level of at least one additional marker of PKD. For example, the determining in (b) can include contacting the sample with antibodies that bind specifically to AMBP protein, and optionally antibodies that bind specifically to the at least one additional marker of PKD. In some embodiments, (b) includes determining the protein level of the additional marker(s) of PKD of at least one of PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6.

In any of these methods, a control level can be a level, e.g., a level of AMBP (and optionally, also a level(s) of the at least one additional marker of PKD), in a subject not presenting with one or more symptoms of PKD and/or not diagnosed as having PKD, a level of the at least one marker in a healthy subject or a population of healthy subjects, or a threshold level (e.g., a level above which indicates that the subject has or may have PKD).

Some embodiments further include recording the identification of PKD in the patient in the patient's medical record (e.g., a computer readable medium). Some examples further include informing the patient, the patient's family, and/or the patient's primary care physician or attending physician of the identification of PKD in the patient. Some examples further include informing the patient's insurance provider of the identification of PKD in the patient.

**Methods of Determining the Stage of PKD in a Patient**

Also provided herein are methods of determining the stage of PKD (e.g., ADPKD or ARPKD) in a patient. Skilled practitioners will readily appreciate that determining the stage
of PKD in a patient can be useful, e.g., in designing and administering a proper treatment regimen to treat the patient and thereby obtaining a desirable outcome. Exemplary methods can include: (a) providing a sample including a biological fluid (e.g., urine) or kidney tissue (e.g., kidney biopsy sample) from a patient suspected of having PKD or identified as having PKD; (b) determining a level of AMBP in the sample; and (c) determining the stage of PKD in the patient from the level. In some examples, the methods of determining the stage of PKD in a patient include: (a) providing a sample including a biological fluid (e.g., urine) or kidney tissue (e.g., kidney biopsy sample) from a patient suspected of having PKD or identified as having PKD; (b) determining a level of AMBP and a level of at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) additional marker of PKD (e.g., PCNA, cyclin D1, cyclin D3, MEK, S6, pS6, ERK, pERK, Akt, pAkt, caspase-2, total S6, and RBBP) in the sample; and (c) determining the stage of PKD in the patient from the level of AMBP and the level of one (or the levels of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) of the at least one additional markers of PKD.

In some embodiments, the determining in (b) includes comparing the determined level of AMBP (and optionally the level(s) of the at least one additional marker of PKD) to a range of values for a particular stage of PKD (e.g., stage I, stage II, stage III, stage IV, or stage V) and identifying a subject as having a particular stage of PKD if the level of AMBP (and optionally also the level of one (or the levels of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) of the at least one additional marker of PKD) falls within a range of values for the particular stage of PKD. Some embodiments further include after (c): (d) administering a treatment for stage I, stage II, stage III, stage IV, or stage V PKD to a patient identified as having stage I, stage II, stage III, stage IV, or stage V PKD, respectively. Some embodiments further include after (c): (d) performing one or more assays to confirm the stage of PKD (e.g., imaging one or both kidney(s) in a patient after (c) to confirm the stage of PKD in the patient). Some embodiments further include after (c): (d) hospitalizing a subject identified as having stage IV or stage V PKD. Ranges of levels of AMBP (and optionally also ranges of the level(s) of the at least one additional marker of PKD) in a sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) from a subject having a certain stage of PKD (e.g., stage I, stage II, stage III, stage IV, or stage V PKD) can be determined using methods known in the art. The five stages of PKD are known in the art and descriptions of the stages are available in various
publications. For example, the five stages are described on the Kidney Support webpage (kidney-support.org): stage 1 (emergence stage), stage 2 (growth stage), stage 3 (enlargement or swelling stage), stage 4 (cyst rupture stage), and stage 5 (end stage).

In some embodiments, step (b) includes determining a level (or levels of two, three, four, five, six, or seven) of additional markers of PKD selected from the group of: PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the stage of PKD is determined from the level of AMBP and a level (or levels of two, three, four, five, six, or seven) of the additional markers of PKD.

In some examples, the determining the levels of AMBP and optionally, the determining of the level of at least one additional biomarker of PKD in (b) includes determining the protein level of AMBP and optionally, the protein level of at least one additional marker of PKD. For example, the determining in (b) can include contacting the sample with antibodies that bind specifically to AMBP protein, and optionally antibodies that bind specifically to the at least one additional marker of PKD. In some embodiments, (b) includes determining the protein level of the additional marker(s) of PKD of at least one of PCNA, cyclin D3, MEK, and phosphorylated S6.

Methods of Monitoring PKD

Also provided are methods of monitoring a PKD patient (e.g., a ADPKD patient or an ARPKD patient). Monitoring can be useful, e.g., for observing a PKD patient's reaction to a given treatment and providing the skilled practitioner with information as to whether the treatment should be continued, modified, or stopped. Exemplary methods can include: (a) providing a first sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) obtained from the PKD patient; (b) determining a level of AMBP in the first sample; (c) providing a second sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) from the PKD patient after step (b) or after the first sample was obtained from the patient, and determining a level of AMBP in the second sample; and (d) identifying the patient as having improving or static PKD if the level in the second sample is not higher than the level in the first sample (or optionally, identifying the subject as having worsening or progressing PKD if the level in the second sample is higher than the level in the first sample).

Also provided are methods of monitoring a PKD patient that include: (a) providing a first sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy
sample) obtained from the PKD patient; (b) determining a level of AMBP and a level(s) of at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) additional marker of PKD (e.g., PCNA, cyclin D1, cyclin D3, MEK, S6, pS6, ERK, pERK, Akt, pAkt, caspase-2, total S6, and RBBP) in the first sample; (c) providing a second sample including a biological fluid (e.g., urine) or kidney tissue (e.g., a kidney biopsy sample) from the PKD patient after step (b) or after the first sample was obtained from the patient, and determining a level of AMBP and a level(s) of the at least one additional marker of PKD in the second sample; and (d) identifying the patient as having improving or static PKD if (i) the AMBP level in the second sample is not higher than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) of the at least one additional marker of PKD in the second sample is not higher than the level(s) of the at least one additional marker of PKD in the first sample (or optionally, identifying the subject as having worsening or progressing PKD if (i) the AMBP level in the second sample is higher than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen) of the at least one additional marker of PKD in the second sample is higher than the level of the at least one additional marker(s) of PKD in the first sample).

In some embodiments, the steps (b) and (c) include determining the level of one (or the levels of two, three, four, five, or six) additional marker(s) of PKD selected from the group of: PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the patient is identified as having improving or static PKD if (i) the AMBP level in the second sample is not higher than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, or six) of the additional marker(s) of PKD in the second sample is not higher than the level(s) of the at least one additional marker of PKD in the first sample (or optionally, the patient is identified as having worsening or progressing PKD if (i) the AMBP level in the second sample is higher than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, or six) of the additional marker(s) of PKD in the second sample is higher than the level(s) of the at least one additional marker of PKD in the first sample).

In some embodiments, the steps (b) and (c) include determining the level of one (or the levels of two, three, four, five, or six) additional marker(s) of PKD selected from the group of: PCNA, cyclin D1, cyclin D3, MEK, S6, and pS6, and the patient is identified as having improving or static PKD if (i) the AMBP level in the second sample is not higher than
the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, or six) of the additional marker(s) of PKD in the second sample is not higher than the level(s) of the at least one additional marker of PKD in the first sample (or optionally, the patient is identified as having worsening or progressing PKD if (i) the AMBP level in the second sample is higher than the AMBP level in the first sample, and (ii) the level of one (or the levels of two, three, four, five, or six) of the additional marker(s) of PKD in the second sample is higher than the level(s) of the at least one additional marker of PKD in the first sample).

In some examples, the determining the levels of AMBP and optionally, the determining of the level of at least one additional biomarker of PKD in (b) and (c) includes determining the protein levels of AMBP and optionally, the protein level(s) of at least one additional marker of PKD. For example, the determining in (b) and (c) can include contacting the samples with antibodies that bind specifically to AMBP protein, and optionally antibodies that bind specifically to the at least one additional marker of PKD. In some embodiments, (b) and (c) include determining the protein level of the additional marker(s) of PKD of at least one (e.g., two, three, or four) of PCNA, cyclin D3, MEK, and phosphorylated S6).

Some embodiments further include after (d): (e) administering the same treatment (e.g., any of the exemplary treatments of PKD described herein or known in the art) to a patient identified as having improving or static PKD. For example, the administering in (e) can be the administration of a GCS inhibitor (e.g., (S)-quinuclidin-3-yl (2-(4′-(2-methoxyethoxy)-1, t-biphenyl)-4-yl)propan-2-yl)carbamate; 4-fluoro-1-(5-fluoro-4-(4-((2methoxyethoxy)methyl) phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxy methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(methoxy methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-
ypiperidine-4-carboxamide; 4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-
methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide); carbamic acid, N-[1-
[2-(4-fluorophenyl)-4-thiazolyl]-l-methylethyl]-, (3S)-l-azabicyclo[2.2.2]oct-3-yl ester (as
represented by Formula II); or quinuclidin-3-yl (2-(4'-fluoro-[1, r-biphenyl]-3-yl)propan-2-
yl)carbamate (as represented by compound of Formula III).

Some embodiments of any of the methods further include a step of selecting a patient
having PKD or diagnosing a patient having PKD (prior to step (a)) (e.g., using any of the
exemplary methods of diagnosing PKD described herein). The patient in any of these
methods can be any of the patients described herein. Some embodiments of any of the
methods further include obtaining the first and/or second samples from the PKD patient.

Some embodiments further include recording the improving or static PKD status (or
alternatively the worsening or progressing status) of the patient in the patient's medical
record (e.g., a computer readable medium). Some examples further include informing the
patient, the patient's family, and/or the patient's primary care physician or attending
physician of improving or static PKD status (or alternatively the worsening or progressing
status) of the patient. Some embodiments further include authorization of a refill of a
treatment administered to the patient between the time points when the first and second
samples were obtained from the patient, when the patient has been identified as having
improving or static PKD (or alternatively further include authorization not to refill a
treatment administered to the patient between the time points when the first and second
samples were obtained from the patient, when the patient has been identified as having
worsening or progressing PKD). Some embodiments include discharging a subject from an
inpatient facility (e.g., hospital) or decreasing inpatient treatments (e.g., dialysis) based on
identification of the subject as having improving or static PKD (or include contuing
inpatient treatment (e.g., hospitalization) or increasing inpatient treatments (e.g., dialysis) of
the subject based on identification of the subject as having worsening or progressing PKD).

The difference in time between when the first and second samples are obtained from
the patient can be, e.g., between 1 week and 40 weeks, between 1 week and 30 weeks,
between 1 week and 20 weeks, between 1 week and 12 weeks, between 1 week and 8 weeks,
between 1 week and 4 weeks, between 1 week and 2 weeks, between 2 weeks and 12 weeks,
between 2 weeks and 8 weeks, or between 2 weeks and 4 weeks.
Kits

Also provided herein are kits that consist essentially of or consist of an antibody that specifically binds to AMBP protein, and at least one (e.g., two, four, five, six, seven, eight, nine, ten, eleven, or twelve) antibody that specifically binds to an additional protein marker of PKD. For example, the kits can consist essentially of or consist of an antibody that specifically binds to AMBP protein and at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve) antibody selected from the group consisting of: an antibody that specifically binds to PCNA, an antibody that specifically binds to cyclin D1, an antibody that specifically binds to cyclin D3, an antibody that specifically binds to MEK, an antibody that specifically binds to S6, an antibody that specifically binds to pS6, an antibody that specifically binds to ERK, an antibody that specifically binds to pERK, an antibody that specifically binds to Akt, an antibody that specifically binds to pAkt, an antibody that specifically binds to caspase-2, and an antibody that specifically binds to retinoblastoma binding protein (RBBP). In some examples, any combination of the antibody that specifically binds to AMBP protein and/or the at least one antibody that specifically binds to an additional protein marker of PKD are labeled (e.g., with a radioisotope, a fluorophore, or a quencher).

Some exemplary kits further include one or more positive control recombinant proteins (e.g., an isolated recombinant AMBP, PCNA, cyclin D1, cyclin D3, MEK, S6, pS6, ERK, pERK, Akt, pAkt, caspase-2, and RBBP). In some examples, the antibody that specifically binds to AMBP and the antibody that specifically binds to the at least one additional protein marker of PKD are covalently attached to a solid surface (e.g., a chip, a bead, or a membrane) by the Fc domain.

Some kits further include a sample including a biological fluid (e.g., a sample including a biological fluid, kidney tissue, or kidney cells) from a PKD patient (e.g., a PKD patient with a known severity of PKD) or an animal model of PKD (e.g., any of the animal models described in the Examples). Such samples are useful, e.g., as positive controls.

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.
EXAMPLES

Example 1. Identification of AMBP as a Marker of PKD and Levels of AMBP in Human PKD Patients and Two Different Mouse Models of PKD

A set of experiments was performed to determine whether AMBP is an accurate marker of PKD in humans and in two different animal models of PKD.

Materials and Methods

Animals and Urine Collection

C57BL/6J jck/+ mice were maintained for mating. Cystic jck/jck mice were genotyped as previously described (Smith et al., J. Am. Soc. Nephrol. 17:2821-2831, 2006). Pdkl conditional knockout mice were generated as described previously (Natoli et al., Nature Med. 16:788-792, 2010). The Pdkl gene was deleted by inducing the Cre recombinase activity with tamoxifen delivered in sunflower oil (Sigma-Aldrich, St. Louis, MO), on postnatal day 1 with 100 mg/kg.

Patient Sample Collection

Normal human patients and samples from human ADPKD patients were purchased from the National Disease Research Institute (NDRI). Urine samples from human ADPKD patients were collected at the University of Toronto. Briefly, mid void morning urine samples were collected and stabilized with Complete Proteinase Inhibitor Cocktail (Roche). The urine was centrifuged at 2000 x g for 10 minutes to remove cellular debris and stored at -80 °C. Total kidney volume (TKV) was quantified in ADPKD patients by magnetic resonance imaging (MRI) (without galolinium) (Table 1).
Table 1. Profile of Patients Representing Early and Late Stage ADPKD with TKV 300-2000 nLs measured by MRI.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age at MRI (yr)</th>
<th>Gender</th>
<th>Total Kidney Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>f</td>
<td>256</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>f</td>
<td>467</td>
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<tr>
<td>3</td>
<td>42</td>
<td>f</td>
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<tr>
<td>4</td>
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<td>f</td>
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<tr>
<td>5</td>
<td>44</td>
<td>f</td>
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<tr>
<td>6</td>
<td>46</td>
<td>f</td>
<td>1581</td>
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<tr>
<td>7</td>
<td>61</td>
<td>f</td>
<td>1689</td>
</tr>
<tr>
<td>8</td>
<td>41</td>
<td>m</td>
<td>1972</td>
</tr>
</tbody>
</table>

**LC-MS/MS Sample Preparation**

Proteoseek HAS/IgG antibody based removal kit (Pierce, Rockford, IL) was used to deplete albumin and the major subclasses of IgG from 100 µg of the urine samples. The depleted samples were precipitated in 10% (v/v) TCA (EMD Chemical) for 2 hours on ice. The precipitate was pelleted at 14000 x g for 15 minutes and the supernatant was discarded. The pellet was washed with 200 µL acetone (-80 °C), vortexed, and re-pelleted at 14000 x g for 10 minutes, and the supernatant discarded. The pellet was resuspended, reduced, alkylated, and trypsin digested using ProteaseMax degradable surfactant, and the described Insolution protocol (Promega). The degraded surfactant was removed by centrifuging the sample at 14,000 x g for 15 minutes, and the supernatant transferred to a Total Recovery Vial (Waters) for nLC-MS/MS analysis.

**nanoUPLC-MS**

The peptide digest was desalted, with an online Symmetry C18 trap column (180 µm x 20 mm x 5 µm (Waters) at a flow rate of 15 µL/min using 99% buffer A (0.1% v/v formic acid in water) and 1% buffer B (0.1% v/v formic acid in acetonitrile) for 3 minutes. Peptides were eluted over a 90 minute gradient on a C18 BEH 100 µm x 100 µm, 1.7 µm (Waters) column. The nanoAcuity UPLC was coupled to a Synapt G1 (Waters) through a TaperTip emitter (New Objective). Data independent acquisition mode (MS²) in continuum format was used to analyze eluting peptides in the range of 200-3000 m/z. Mass accuracy was maintained using lockspray calibrant ([Glul]-fibrinopeptide B ([M+2 H]²⁺, 785.84206 m/z, Genzyme, Framingham, MA).
Data Analysis

MSE raw data was automatically smoothed, background subtracted, centered, deisotoped, charge state reduced, and mass corrected with Protein Lynx Global Server v2.4 (Waters). Processed data was searched against the human IPI protein database v3.131. Filtering criteria were set to include only proteins with >95% confidence scores. Label free quantitation using Expression Analysis (Waters) was also used to rank order those proteins with a differential expression of >1.5 fold over the control.

Western Blot (Immunoblot) Analysis

Kidney samples were homogenized in RIPA buffer (Boston BioProducts) including 1 mM DTT, 5 mM EDTA, 2 mM NaF, 1 mM Na3VO4 (all supplied by Sigma-Aldrich), Pefabloc SC and Complete Proteinase Inhibitor Cocktail (both from Roche Applied Scine). Protein concentrations were determined by BCA protein assay (Pierce). Urine samples were briefly centrifuged at 3000 RPM for 10 minutes at 4 °C (Beckman Coulter Allegra 6A) to remove cellular debris. An equal volume of urine was diluted in 5X Laemmli Buffer (15% SDS, 0.575 M sucrose, 0.325 M Tris, pH 6.8, 5% beta-mecaptoethanol and 0.002% bromophenol blue). Equal amounts of protein and urine were loaded onto 4-14% NuPage Bis-Tris gels following the manufacturer's protocols (Invitrogen). Membranes were blocked with 5% nonfat milk in Tris-buffered saline (TBS) including 0.1% Tween-20 and incubated with primary antibodies overnight at 4 °C. Primary antibodies were detected with horseradish peroxidase-labeled secondary antibodies (Promega). Immunoreactive proteins were detected by enhanced chemiluminescence (GE Healthcare). Primary antibodies to the following proteins were used: a-1-microglobulin and β-actin (Abeam), and GAPDH (US Biological).

Quantitative RT-PCR Analysis

RNA extraction was performed by homogenizing kidneys in TRIzol reagent in the presence of 5 µg glycogen (Invitrogen) following the manufacturer's instructions. Reverse transcriptase reactions were conducted using extracted RNA with the Superscript III First-Strand Synthesis SuperMix for qRT-PCR following the manufacturer's recommendations (Invitrogen). TaqMan primers were obtained from Applied Biosystems predesigned Taqman Gene Expression assays corresponding to AMBP (Mm00431788_ml). Reactions were
performed using TaqMan Gene Expression Master Mix, run on an Applied Biosystems 7500 Real-Time PCR system and normalized to rodent GAPDH expression.

**Immunofluorescence**

Paraffin-embedded kidney specimens from normal patients and ADPKD patients were obtained from the National Disease Research Institute. Paraffin-embedded kidneys from wild type, *yck*, control, and *Pkdl cKO* were cut in four-micrometer sections and boiled in Antigen Retrieval Solution (DAKO) in a pressure cooker to unmask antigens. The kidney sections were blocked for 1 hour with Protein Block Serum Free (DAKO) overnight at 4 °C. Primary antibodies to *Lotus tetragonolobus* lectin (LTL) (Vector Laboratories) was used at a dilution of 1:1000. Staining was visualized on an Olympus 1X70 microscope with a 20x or 40x objective (Olympus-America). Images were captured with Metamorph Imaging Series software (Molecular Devices Corporation).

**Results**

Urine from eight human patients with ADPKD (seven female and one male) and three normal human patients. The clinical parameters for each human ADPKD patient are listed in Table 1. Urine for each human subject was collected mid-void from three patients with mild disease (TKV < 600 mL) and from five patients with moderately severe ADPKD (TKV > 750 mL). Global urinary proteome profiling using LC-MS/MS was used to compare the protein expression in urine from normal controls to each individual ADPKD sample. The data revealed that α-1-microglobulin/bikunin precursor (AMBP) protein was increased in ADPKD patients and that the levels of AMBP protein correlated with total kidney volume (TKV) measurements. A comparison of the AMBP expression from the least diseased (patient 1) and the most diseased (patient 8) ADPKD patients showed a 30-fold difference in AMBP expression, suggesting that AMBP protein levels increased with increasing severity of ADPKD in humans.

**Analysis of AMBP Expression in Human ADPKD**

gene is regulated by an AlM-specific cis elements and transcription factors. There is evidence that in renal tubular cells, there is a lack of cleavage of AMBP protein (Grewal et al, *Biochem. J.* 387:609-616, 2005).

**Immunoblotting analysis of AMBP protein levels in urine samples from human ADPKD patients** was performed to confirm the LC-MS/MS data. The analysis demonstrated that AMBP is up-regulated in urine samples from human ADPKD patients and levels of AMBP expression correlate with disease progression (as indicated by TKV) ($r^2 = 0.7782$) (Figures 1-3). Sections from normal and human ADPKD patient kidneys were stained with a primary antibody against AIM and proximal tubule marker LTL to determine the localization of AMBP expression in human kidneys. As shown in Figure 4, tubules stained with proximal tubule marker LTL is the site of AMBP protein expression in ADPKD patient kidneys (indicated by arrows), while such expression is not observed in normal patient kidneys. The expression of AMBP protein in ADPKD cystic fluid was confirmed using cyst fluid from three different cysts in a single human ADPKD patient.

These data indicate that AMBP is a marker of PKD.

**Assessment of AMBP Expression in the** *jck* **Model of PKD**

A *jck* mouse model of PKD (Smith et al., *J. Am. Soc. Nephrol.* 17:2821-2831, 2006) was used to assess the expression of AMBP. *Jck* mice are characterized by development of moderately progressive renal cystic disease. Kidneys from the *jck* mouse model are enlarged by day 26 after birth and have multiple cysts. By day 64, little normal tissue remains and there is significantly increased number and size of cysts. Renal function in *jck* mice, as measured by serum creatinine and blood urea nitrogen, progressively elevates over time. In *jck* mice, cysts originate early in disease from the collecting ducts and over the progression of cytogenesis, cysts develop from the distal tubules and loop of Henle. Cysts originating from the proximal tubules are not detected in *jck* mice (Smith et al, *J. Am. Soc. Nephrol.* 17:2821-2831, 2006).

The data in Figure 5 show that, a 64-day-old *jck* mouse has a 60-fold higher level of AMBP gene expression compared to a wild type mouse control. These data are supported by corresponding immunoblots, which also show a significant elevation of AMBP protein levels in kidneys from 50- and 60-day-old *jck* mice as compared to wild type mice (Figures 6 and 7). Immunofluorescence micrographs of kidney sections from *jck* mice at 26, 50, and 60 days after birth and wild type mice at 64 days after birth show increasing expression of
AMBP protein in proximal tubules and glomeruli over disease progression in jck mice, while little AMBP protein expression is observed in the 64-day old wild type mouse (Figure 8).

In a next set of experiments, AMBP protein levels were measured in urine samples from jck mice at different stages of disease progression. Urine was collected consecutively over a 5-week period from 5jck mice with a range of disease severity measured by kidney to body weight ratio (final K/BW) at 64 days after birth in the 6.95 to 8.72 range. The immunoblot data show that an elevation in AMBP protein levels occurs starting at day 33 to day 41 after birth and increases progressively in jck mice, and more severe disease is characterized by earlier up-regulation of urinary AMBP protein levels and higher AMBP protein levels at day 64 after birth in jck mice (Figures 9 and 10). These data suggest that AMBP protein levels (e.g., urine or kidney tissue AMBP levels) can be used to determine the stage or severity of PKD.

**AMBP Expression in an Orthologous Model of ADPKD**

Experiments were performed using an Pkdl cKO (conditional knockout) mouse (Natoli et al, *Nature Med.* 16:788-792, 2010) in order to investigate expression of AMBP in an orthologous mouse model of PKD. In this model, inactivation of Pkdl on postnatal day 1 results in significant cystogenesis evident by increased kidney to body weight ratio, cyst percentage, and blood urea nitrogen (BUN) (Natoli et al, *Nature Med.* 16:788-792, 2010).

The progressive enlargement of cysts in the cortical and medullary regions occurs in two phases, an initial rapid cyst growth (18-26 days of age) followed by a slower growth rate.

Immunoblot analysis was used to determine the AMBP protein levels over time in a Pkdl cKO mouse. The data show AMBP protein levels increase in kidney samples from Pkdl cKO mice over time (compare the level of expression at day 64 after birth to the level of expression at day 26 after birth) (Figure 11). Urine was collected consecutively over a 5-week period from Pkdl mice with a range of disease severity (as measured by cyst percentage) (final Cyst% at 64 days after birth in the 23.3 to 39.6 range) to further investigate urinary levels of AMBP protein in Pkdl cKO mice. The data in Figures 13 and 14 show that levels of AMBP protein in urine is increased in Pkdl mice between 26 to 33 days followed by a relatively slow increase in levels till day 64 after birth (used as a terminal time point for this study). Similar to jck mice kidneys, immunofluorescent analysis of Pkdl cKO kidney sections showed elevation of AMBP in proximal tubules (Figure 12).
Example 2. AMBP Expression in Preclinical Models of PKD After Therapeutic Intervention

Previously it has been shown that cyclin dependent kinase inhibitors (CDKi) and glucosylceramide synthase inhibitor (GCSi) reduces cystic parameters in mouse models of PKD (Natoli et al., *Nature Med.* 16:788-792, 2010; Bukanov et al, *Nature* 444:949-952, 2006; Bukanov et al, *Cell Cycle* 11:4040-4046, 2012). A set of experiments were performed to determine whether kidney and urinary AMBP expression in *jck* mice would be reduced after successful therapeutic intervention with CDKi Roscovitine and S-CR8, and a GCSi (Genz-123346).

Materials and Methods

The materials and methods used in this set of experiments is the same as for Example 1, except for the mouse model treatments described below.

*Animal Treatments*

Roscovitine was administered *ad libitum* to *jck* mice from 26 to 64 days of age by mixing in powdered 5053 diet at 0.2%. The GCSi (Genz-123346) was administered *ad libitum* to *jck* mice from day 26 to day 64 after birth by mixing in powdered 5053 diet at 0.225% (Natoli et al., *Nature Med.* 16:788-792, 2010). Chronic and pulse treatment with S-CR8 was performed by daily intraperitoneal injection with 24 mg/kg starting from day 26 after birth. The schedules for S-CR8 treatment are shown in Figure 18. Urine was collected for 24 hours consecutively (at day 26, 33, 41, 48, and 64 after birth) over a 5-week period from animals with a range of disease severity measured by kidney to body weight ratio (K/BW) and cystic percentage (C%).

Results

*Treatment with CDKInhibitors Roscovitine and S-CR8 in Jck Mice*

A first set of experiments was performed in *jck* mice to determine whether administration of roscovitine would decrease both AMBP levels and kidney volume. The data show that oral administration of 0.2% roscovitine between day 26 to day 64 after birth (Figure 15) results in both a decrease in kidney volume (Figure 16) and a decrease in AMBP protein levels in both urine and kidney samples from *jck* mice at day 64 after birth (Figure...
17), as compared to the same parameters measured in a 64-day-old jck mice administered only a vehicle between day 26 to day 64 after birth.

A second set of experiments was performed in jck mice to determine a second CDK inhibitor, S-CR8, would decrease both AMBP levels and kidney volume. In these experiments, the jck animals were administered one of the following treatment schedules starting at day 26 after birth: (A) daily intraperitoneal injection with 24 mg/kg S-CR8 for five weeks; (B) daily intraperitoneal injection with 24 mg/kg S-CR8 for three weeks, and two weeks with no treatment; and (D) daily intraperitoneal injection with 24 mg/kg S-CR8 for one week and four weeks with no treatment (Figure 18). As a control, jck animals were administered a vehicle between day 26 to day 64 after birth.

The data show that daily intraperitoneal injection of 24 mg/kg S-CR8 (in all tested treatment schedules) resulted in a decrease in cyst volume (Figure 19) and a corresponding decrease in urine AMBP protein levels (Figures 20 and 21). These data show that decreasing levels of AMBP can indicate efficacy of a treatment for PKD in a subject.

**Treatment with a GCS Inhibitor in Jck Mice**

An additional set of experiment was performed to test the effect of administration of a GCS inhibitor, Genz-123346, on AMBP levels in urine and kidney samples of jck mice. The data show that daily administration of 0.225% Genz123346 to jck mice between day 26 to day 64 after birth results in a decrease in both urine and kidney tissue samples at day 64 after birth (Figure 22) as compared to the corresponding levels in a 64-day-old control jck mouse administered a vehicle between day 26 to day 64 after birth.

**OTHER EMBODIMENTS**

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.
WHAT IS CLAIMED IS:

1. A method of determining the efficacy of treatment for polycystic kidney disease (PKD) in a patient, the method comprising:
   (a) providing a first sample comprising a biological fluid obtained from a PKD patient;
   (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the first sample;
   (c) administering a PKD treatment to the patient;
   (d) providing a second sample comprising a biological fluid from the patient after step (c) and determining a level of AMBP in the second sample; and
   (e) identifying the administered treatment as effective if the level in the second sample is lower than the level in the first sample.

2. The method of claim 1, wherein the PKD patient has autosomal dominant PKD.

3. The method of claim 1, wherein the PKD patient has autosomal recessive PKD.

4. The method of claim 1, wherein the first and second samples comprise urine.

5. The method of claim 1, wherein the PKD treatment comprises a glucosyl ceramide synthase (GCS) inhibitor.

6. The method of claim 5, wherein the GCS inhibitor is selected from the group consisting of:
   (S)-quinuclidin-3-yl (2-(4′-(2-methoxyethoxy)-[1,1′-biphenyl]-4-yl)propan-2-yl)carbamate;
   4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;
   4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;
   4-fluoro-1-(4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;
4-fluoro-l-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)(2-(4’-fluoro-[l[2-(4-fluorophenyl)-4-thiazolyl]-l-methylthethyl]-, (3S)-1-azabicyclo[2.2.2]oct-3-yl ester.

7. The method of claim 5, further comprising: (f) administering to the patient additional doses of GCS inhibitor if the treatment is identified as being effective.

8. The method of claim 7, wherein the additional doses of GCS inhibitor comprise (S)-quinuclidin-3-yl (2-(4’-(2-methoxyethoxy)-[l-l’-biphenyl]-4-yl)propan-2-yl)carbamate;

4-fluoro- l-(5-fluoro-4-(4-(2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro- l-(4-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;
4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;
4-fluoro-1-(4-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;
4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quinuclidin-3-yl)piperidine-4-carboxamide;
4-fluoro-1-(4-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;
quinoxalin-3-yl(2-(4'-fluoro-[1,r-biphenyl]-3-yl)propan-2-yl)carbamate; or carbamic acid, N-[1-[2-(4-fluorophenyl)-4-thiazolyl]-l-methylethyl]-, (3S)-1-azabicyclo[2.2.2]oct-3-yl ester.

9. The method of claim 1, wherein the PKD treatment comprises a CDK inhibitor.

10. The method of claim 9, wherein the CDK inhibitor is R-roscovitine or S-CR8.

11. The method of claim 9, further comprising: (f) administering to the patient additional doses of CDK inhibitor if the treatment is identified as being effective.

12. The method of claim 11, wherein the additional doses of CDK inhibitor comprise R-roscovitine or S-CR8.

13. The method of claim 1, wherein determining the level of AMBP in (b) and (d) comprise determining the level of AMBP protein.

14. The method of claim 13, wherein (b) and (d) comprise contacting the sample with an antibody that binds specifically to AMBP protein.

15. A method of determining the stage of polycystic kidney disease (PKD) in a patient comprising:
(a) providing a sample comprising a biological fluid from a patient suspected of having PKD or identified as having PKD;
Ati

(b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the sample; and
(c) determining the stage of PKD in the patient from the level.

16. The method of claim 15, wherein the PKD is autosomal dominant PKD.

17. The method of claim 15, wherein the PKD is autosomal recessive PKD.

18. The method of claim 15, wherein the sample comprises urine.

19. The method of claim 15, further comprising: (d) administering a treatment for stage I, stage II, stage III, stage IV, or stage V PKD to a patient identified to have stage I, stage II, stage III, stage IV, or stage V PKD, respectively.

20. The method of claim 19, further comprising: (d) imaging one or both kidney(s) in the patient after (c) to confirm the stage of PKD in the patient.

21. The method of claim 15, wherein determining the level of AMBP in (b) comprises determining the level of AMBP protein.

22. The method of claim 21, wherein (b) comprises contacting the sample with an antibody that binds specifically to AMBP protein.

23. A method of determining the stage of polycystic kidney disease (PKD) in a patient comprising:
   (a) providing a sample comprising kidney tissue from a patient suspected of having PKD or identified as having PKD;
   (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the sample; and
   (c) determining the stage of PKD in the patient from the level.

24. The method of claim 23, wherein the PKD is autosomal dominant PKD.
25. The method of claim 23, wherein the PKD is autosomal recessive PKD.

26. The method of claim 23, further comprising: (d) administering a treatment for stage I, stage II, stage III, stage IV, or stage V PKD to a patient identified to have stage I, stage II, stage III, stage IV, or stage V PKD, respectively.

27. The method of claim 23, further comprising: (d) imaging one or both kidney(s) in the patient after (c) to confirm the stage of PKD in the patient.

28. The method of claim 23, wherein determining the level of AMBP in (b) comprises determining the level of AMBP protein.

29. The method of claim 28, wherein (b) comprises contacting the sample with an antibody that binds specifically to AMBP protein.

30. A method of diagnosing polycystic kidney disease (PKD) in a patient comprising:
   (a) providing a sample comprising a biological fluid from a patient suspected of having PKD;
   (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the sample; and
   (c) identifying the patient as having PKD if the level is elevated as compared to a control level.

31. The method of claim 30, wherein the PKD is autosomal dominant PKD.

32. The method of claim 30, wherein the PKD is autosomal recessive PKD.

33. The method of claim 30, wherein the sample comprises urine.

34. The method of claim 30, further comprising: (d) administering a PKD treatment to the patient.
35. The method of claim 34, wherein the PKD treatment comprises a glucosyl ceramide synthase (GCS) inhibitor.

36. The method of claim 35, wherein the GCS inhibitor is selected from the group consisting of:

(S)-quinuclidin-3-yl (2-(4′-(2-methoxyethoxy)-[1,r-biphenyl]-4-yl)propan-2-yl)carbamate;

4-fluoro-1-(5-fluoro-4-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-methoxyethoxy)methyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-azabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quINUCLIDIN-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-((2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

quinuclidin-3-yl (2-(4′-fluoro-[1,9′-biphenyl]-3-yl)propan-2-yl)carbamate; and carbamic acid, N-[l-[2-(4-fluorophenyl)-4-thiazolyl]-l-methylethyl]- (3S)-1-azabicyclo[2.2.2]oct-3-yl ester.

37. The method of claim 34, wherein the PKD treatment comprises a CDK inhibitor.

38. The method of claim 37, wherein the CDK inhibitor is R-roscovitine or S-CR8.
39. The method of claim 30, further comprising: (d) imaging one or both kidney(s) in the patient.

40. The method of claim 30, wherein the control level is a threshold level or a level in a healthy subject or a population of healthy subjects.

41. The method of claim 30, wherein determining the level of AMBP in (b) comprises determining the level of AMBP protein.

42. The method of claim 41, wherein (b) comprises contacting the sample with an antibody that binds specifically to AMBP protein.

43. A method of diagnosing polycystic kidney disease (PKD) in a patient comprising:
   (a) providing a sample comprising kidney tissue from a patient suspected of having PKD;
   (b) determining a level of α-1-microglobulin/bikunin precursor (AMBP) in the sample; and
   (c) identifying the patient as having PKD if the level is elevated as compared to a control level.

44. The method of claim 43, wherein the PKD is autosomal dominant PKD.

45. The method of claim 43, wherein the PKD is autosomal recessive PKD.

46. The method of claim 43, further comprising: (d) administering a PKD treatment to the patient.

47. The method of claim 46, wherein the PKD treatment comprises a glucosyl ceramide synthase (GCS) inhibitor.
48. The method of claim 47, wherein the GCS inhibitor is selected from the group consisting of:

(S)-quinuclidin-3-yl (2-(4’-(2-methoxyethoxy)-[1,r-biphenyl]-4-yl)propan-2-yl)carbamate;

4-fluoro-1-(5-fluoro-4-(2-methoxyethoxy)methyl)pyrimidin-2-yl)-N-(4-methyl-1-aazabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(2-methoxyethoxy)methyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-aazabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(methoxymethyl)phenyl)pyrimidin-2-yl)-N-(3-methylquinuclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(2-methoxyethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-aazabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(quINUclidin-3-yl)piperidine-4-carboxamide;

4-fluoro-1-(4-(2-fluoroethoxy)phenyl)pyrimidin-2-yl)-N-(4-methyl-1-aazabicyclo[3.2.2]nonan-4-yl)piperidine-4-carboxamide;

quinuclidin-3-yl (2-(4’-fluoro-[1,r-biphenyl]-3-yl)propan-2-yl)carbamate; and carboxylic acid, N-[l-(2-(4-fluorophenyl)-4-thiazolyl)-l-methylethyl]-, (3S)-1-aazabicyclo[2.2.2]oct-3-yl ester.

49. The method of claim 46, wherein the PKD treatment comprises a CDK inhibitor.

50. The method of claim 49, wherein the CDK inhibitor is R-roscovitine or S-CR8.

51. The method of claim 43, further comprising: (d) imaging one or both kidney(s) in the patient.
52. The method of claim 43, wherein the control level is a threshold level or a level in a healthy subject or a population of healthy subjects.

53. The method of claim 43, wherein determining the level of AMBP in (b) comprises determining the level of AMBP protein.

54. The method of claim 53, wherein (b) comprises contacting the sample with an antibody that binds specifically to AMBP protein.

55. A method of monitoring a polycystic kidney disease (PKD) patient, the method comprising:
   (a) providing a first sample comprising a biological fluid obtained from a PKD patient;
   (b) determining a level of α1-microglobulin/bikunin precursor (AMBP) in the first sample;
   (c) providing a second sample comprising a biological fluid from the patient after step (b) and determining a level of AMBP in the second sample; and
   (d) identifying the patient as having improving or static PKD if the level in the second sample is not higher than the level in the first sample.

56. The method of claim 55, wherein the PKD patient has autosomal dominant PKD.

57. The method of claim 55, wherein the PKD patient has autosomal recessive PKD.

58. The method of claim 55, wherein the first and second samples comprise urine.

59. The method of claim 55, further comprising: (e) administering the same treatment to a patient identified as having improving or static PKD.

60. The method of claim 55, wherein determining the level of AMBP in (b) and (c) comprise determining the level of AMBP protein.
61. The method of claim 60, wherein (b) and (c) comprise contacting the sample with an antibody that binds specifically to AMBP protein.

62. A kit consisting essentially of:
   an antibody that specifically binds to α-1-microglobulin/bikunin precursor (AMBP) protein; and
   an antibody that specifically binds to an additional protein marker of polycystic kidney disease.
Figure 7

Figure 8
Figure 15

Figure 16

Figure 17
### Figure 21

Bar chart showing quantification of AMBP (AU) with different treatments labeled as wt, Veh, A, B, and D. The chart indicates a 2.8-fold increase for treatment A compared to wt and a 2.4-fold increase for treatment B compared to wt.

### Figure 22

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### A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

### Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- EPO-Internal
- BIOSIS
- EMBASE
- WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>AU 2012 318 734 Al (CELMATIX INC) 17 April 1 2014 (2014-04-17) Table 3; claims 1, 7, 9; pg 54, 1 12-20</td>
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- Date of the actual completion of the international search: 25 January 2017
- Date of mailing of the international search report: 17/02/2017

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