Methods

The present invention relates to treatment and diagnosis of patients with polycystic kidney disease (PKD).

PKD is reviewed in detail in Guay-Woodford (2003) Am J Physiol Renal Physiol 285, F1034-F1049. Renal tubular cysts develop in several inherited human disorders. Among these, the polycystic kidney diseases (PKD) are one of the leading causes of endstage renal disease in children and adults (Gabow, (1993) N Engl J Med 329, 332-342). Autosomal dominant polycystic kidney disease (ADPKD) occurs in 1:1,000 individuals, primarily as the result of mutations in one of two genes, *PKD1* or *PKD2* (Mochizuki et al (1996) Science 272, 1339-1342; The American PKD1 Consortium (1995) Hum Mol Genet 4, 575-582; The European Polycystic Kidney Disease Consortium (1994) Cell 77, 881-894; The International Polycystic Kidney Disease Consortium (1995) Cell 81, 289-298). Rarer forms include autosomal recessive PKD (ARPKD), resulting primarily from mutations in a single gene, *PKHD1*, and nephronophthisis with high mortality and morbidity in children.

The principal pathological manifestations in PKD involve *1*) the formation of epithelial-lined cysts throughout the nephron in ADPKD and predominantly in the collecting duct in ARPKD; *2*) alterations in cell polarity; and *3*) changes in extracellular matrix composition. In addition to the renal cystic disease, ADPKD is associated with cyst formation in other epithelial organs, most notably the liver and pancreas, as well as connective tissue defects, such as intracranial aneurysms, aortic dissection, cardiac valve abnormalities, and abdominal wall hernias (Perrone et al (1997) Kidney Int 51, 2022-2036). In comparison, the ARPKD phenotype is expressed almost exclusively in the kidney and liver, with the latter lesion

involving biliary dysgenesis and portal tract fibrosis (D'Agata et al (1994) Semin Liver Dis 14, 265-272).

Efforts to elucidate the mechanisms that underlie PKD pathogenesis have been greatly enhanced by studies in experimental systems, most notably murine (mouse and rat) models of PKD. Numerous mouse and rat PKD models have been described in which the mutant phenotypes closely resemble human PKD with respect to cyst morphology, cyst localization, and disease progression (reviewed in Gretz et al (1996) Nephrol Dial Transplant 11, 46-51; Schieren et al (1996) Nephrol Dial Transplant 11, 38-45). Some of these models are the result of spontaneous mutations, whereas others were engineered through chemical mutagenesis, transgenic technologies, or gene-specific targeting in mouse orthologs of human PKD genes. These murine models share common pathogenic features with human PKD. These include *1*) dysregulated epithelial cell proliferation and differentiation; *2*) alterations of tubular basement membrane constituents and the associated extracellular matrix; *3*) abnormalities of epithelial cell polarity with apical mislocalization of key receptors and enzymes; and *4*) abnormalities in transepithelial fluid transport (reviewed Calvet and Grantham (2001) Semin Nephrol 21, 107-123). These parallel observations in murine models and human PKD prompt the hypothesis that mammalian PKD genes may define common molecular pathways that are involved in cystogenesis and PKD progression.

TAZ (transcriptional coactivator with PDZ-binding motif) is a recently described transcriptional coactivator factor with binding sites for 14-3-3 proteins, PDZ proteins and a WW domain (Kanai et al (2000) EMBO J 19, 6778-6791). TAZ is known to bind to 14-3-3, a conserved family of ~30-kDa proteins that bind to a large number of intracellular phosphoproteins involved in signal transduction to regulate differentiation, cell cycle

progression, and apoptosis. TAZ has 395 amino acid residues, shares homology with YAP (Yes-associated protein), and contains a single WW domain. It functions as a transcriptional coactivator by binding to the PPXY motif present in targeted transcription factors (Kanai et al, *supra*). The C terminus of TAZ has the highly conserved PDZ-binding motif that helps localization of TAZ into discrete nuclear foci and is essential for TAZ-stimulated gene transcription. PDZ domains are present in a large number of proteins and proteins with PDZ domains may be membrane associated or in some instances end up in the nucleus. The binding of TAZ to 14-3-3 proteins can inhibit nuclear translocation of TAZ and thus inhibit the transcriptional coactivation function (Kanai et al, *supra*).

TAZ is a transcriptional coactivator that binds to the oesteoblast-specific transcriptional factor Cbfa1 (Cui et al (2003) Mol Cell Biol 23, 1004-1013). TAZ has also been shown to act as a transcriptional coactivator of TTF-1, enhancing its transcriptional activity on the mouse surfactant protein C promoter (Park et al (2004) J Biol Chem 279, 17384-17390).

High-level expression of TAZ mRNA has been detected in the mouse heart, lung, liver, and kidney (Kanai et al, *supra*). However, the tissue distribution pattern for TAZ mRNA is slightly different in humans, where it was additionally found in the skeletal muscle, spleen, small intestine, and placenta (Kanai et al, *supra*).

Cui et al, *supra*, present data on the expression of TAZ protein in osteoblasts lining the developing bone and the presence of TAZ mRNA in 7-day-old mouse calvaria that is enriched in osteoblasts and argue that this demonstrates that TAZ is expressed in differentiating osteoblasts. The authors also argue that, since TAZ mRNA is also found in nonmineralizing tissues such as the heart, lung, liver, and muscle, TAZ may be either

functional as a transcriptional coactivator on a more general level or it may be functioning at different capacities in these various tissues.

We have found that inactivation of the TAZ gene leads to a condition resembling human nephronophthisis-medullary cystic kidney disease (NPH-MCKD). $TAZ^{-/-}$ mice have a decreased survival rate and, surprisingly, surviving animals develop kidney cysts at the corticomedullary border. Thus, TAZ is not required for kidney development *per se* but plays a critical role in kidney physiology.

NPH-MCKD has been defined as a group of renal cystic diseases based on common features, including clinical symptoms, macroscopic pathology and renal histology (Hildebrandt, F., and Omram, H. (2001). New insights: nephronophthisis-dedullary cystic kidney disease. Pediatr Nephrol 16, 168-176). Characteristic for NPH-MCKD is the development of renal cysts at the corticomedullary border, which is quite distinct from autosomal dominant and recessive polycystic kidney disease (ADPKD, ARPKD), where cysts are distributed uniformly over the entire organ. Although four genes have been linked to NPH (Hildebrandt et al (1997). Nat Genet 17, 149-153; Otto et al (2002). Am J. Hum Genet 71, 1161-1167; Otto et al (2003) Nat Genet 34, 413-420; Olbrich et al (2003) Nat Genet 34, 455-459; Mollet et al (2002) Nat Genet. 32, 300 – 305), they account for less of 25% of known cases (Dr. F. Hildebrandt, personal communication).

The involvement of TAZ in PKD had not been contemplated before the present disclosure. Moreover, as mentioned above TAZ has a broad profile of expression and coactivates transcription factors involved in diverse processes. It is surprising, therefore, that $TAZ^{-/-}$ mice exhibit such a specific phenotype.

$TAZ^{-/-}$ mice animals can be used as disease models in which to study PKD progression and to aid in identifying agents which may be useful as a therapeutic entity. In contrast to previously described PKD model animals, some $TAZ^{-/-}$ mice survive to adulthood providing an opportunity to determine the effect of test therapeutic compounds over a greater lifespan; this is an important advantage of the present invention over the prior known models.

A first aspect of the invention provides a non-human animal which has a reduced amount of functional TAZ polypeptide and/or TAZ-like polypeptide, or a reduced amount of nucleic acid encoding said polypeptide.

Non-human animals having a reduced amount of functional TAZ polypeptide and/or TAZ-like polypeptide can be used as disease models in which to study PKD progression and to aid in identifying agents which may be useful as a therapeutic entity.

As mentioned above, PKD is a term for a range of disorders in which renal cysts develop. The non-human animals provided by the first aspect of the invention may be of use as model animals in which to study these disorders. Hence an embodiment of this aspect of the invention is wherein the non-human animal develops PKD. By "PKD" we include, in all aspects of the invention, all disorders that develop kidney cysts, including autosomal dominant polycystic kidney disease, autosomal recessive PKD and nephronophthisis including NPH-MCKD. Preferably, in all aspects of the invention, we mean NPH-MCKD.

By "reduced amount of functional" we include that, in comparison to a normal animal of the same species or strain, the animal of the first aspect of the invention has a reduced amount of polypeptide and/or less of the

polypeptide can function in the way that the same polypeptide operates in the comparative animal, or a reduced amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide. For example, the animal of this aspect of the invention may have the same amount of polypeptide *per se*, but a greater proportion of the polypeptide is in a non-functional state. Methods of determining the amount of functional polypeptide or the amount of nucleic acid are provided below. Preferably, "reduced amount of functional" includes 50%, 25%, 10%, 5%, 1%, 0.1% or 0% of the amount of functional polypeptide or nucleic acid in the normal animal.

The non-human animal of the first aspect of the invention may have a reduced amount of functional TAZ polypeptide and/or TAZ-like polypeptide due to an agent which can modify the said polypeptide(s) function being supplied to the animal, for example a compound which acts to prevent polypeptide function or sub-cellular localisation, or a peptide or antibody which can bind to the polypeptide and prevent function or sub-cellular localisation. The non-human animal of the first aspect of the invention may have a reduced amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide due to an agent which can cause or induce degradation of said nucleic acid, for example a ribozyme which can target the nucleic acid, or an antisense molecule which can bind to the nucleic acid. Examples of such agents discussed herein.

Alternatively, the animal may be genetically modified in such a manner as to reduce the amount of functional TAZ polypeptide and/or TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide. Preferably, the animal is a genetically modified.

The term "genetically modified" is well known to those skilled in the art. The term includes animals having introduced native or foreign nucleic acid.

The animal may have had a modification made to its genome or may have been supplied with a nucleic acid which can act without modifying the genome. We also include animals in which one or more native genes has/have been inactivated, as will be discussed further below.

In brief, the first aspect of the invention includes those non-human animals in which a gene encoding TAZ polypeptide and/or TAZ-like polypeptide has a point mutation causing a frame-shift in the coding region of said genes using, for example, chemical mutagens; deletions and/or insertions of the whole or a part of the coding and controlling regions of said gene using, for example, physical mutagens; "knockouts" in which the gene is deleted using, for example, homologous recombination; replacements in which regions of the gene is replaced with gene sequence encoding a different polypeptide sequence using, for example, homologous recombination; antisense regulation of gene expression.

The non-human animal may have a reduced amount of functional TAZ polypeptide and/or TAZ-like polypeptide or a reduced amount of nucleic acid encoding said polypeptide through out the entire animal. Alternatively, there may be a reduction in said polypeptide function or nucleic acid only in some organs or tissues of the animal, for example, kidney, heart, placenta, lung, calvaria, bone, skin and brain. Preferably, the reduction in amount of functional TAZ polypeptide and/or TAZ-like polypeptide or the reduction in the amount of nucleic acid encoding said polypeptide is localised to the kidney. Methods of locally reducing polypeptide function or reducing nucleic acid amounts are discussed below.

The first aspect of the invention includes non-human primates such as baboons, chimpanzees and gorillas, new and old world monkeys as well as other mammals such as cats, dogs, rodents, pigs or sheep, or other animals

such as poultry, for example chickens, fish such as zebrafish, or amphibians such as frogs. A preferred embodiment of this aspect of the invention is wherein the animal is a rodent such as a mouse, rat, hamster, guinea pig or squirrel. Preferably the animal is mouse.

To date human TAZ and mouse TAZ polypeptides have been identified. The polypeptide and polynucleotide sequences of human and mouse TAZ are given in GenBank Accession Numbers NP_056287.1, NM_015472.3 CAC17733 and AJ299430, respectively, and are also provided in Figure 3. By "TAZ polypeptide" we include the human and mouse TAZ polypeptides as well as further homologues, orthologues or paralogues of TAZ.

The human and mouse TAZ polypeptides have significant degrees of homology to YAP (Yes-associated protein; Kanai et al, *supra*) polypeptides. To date human, mouse and chicken YAP polypeptides have been identified. The polypeptide and polynucleotide sequences of human, mouse and chicken YAP are given in GenBank Accession Numbers NP_006097, NM_006106, AAH39125, BC039125, NP_990574 and NM_205243, respectively, and are also provided in Figure 4. By "TAZ-like polypeptide" we include the human, mouse and chicken YAP polypeptides as well as further homologues, orthologues or paralogues of these polypeptides having at least 50% identity to TAZ, preferably at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity to the polypeptide sequence of TAZ.

Methods by which homologues, orthologues or paralogues of polypeptides can be identified are well known to those skilled in the art: for example, *in silico* screening or database mining. Preferably, such polypeptides have at least 40% sequence identity, preferably at least 60%, at least 70%, at least

80%, at least 90% or at least 95% sequence identity to the polypeptide sequence of TAZ or TAZ-like polypeptide.

Methods of determining the percent sequence identity between two polypeptides are well known in the art. For example, the percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

The alignment may alternatively be carried out using the Clustal W program (Thompson *et al.*, (1994) *Nucleic Acids Res* **22**, 4673-80). The parameters used may be as follows:

Fast pairwise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.
Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.
Scoring matrix: BLOSUM.

The term "nucleic acid encoding TAZ or TAZ-like polypeptide" includes both DNA and RNA molecules, including mRNA. By encode we mean that the sequence of bases in the nucleic acid molecule is such that, on transcription and/or translation, it encodes a polypeptide having the sequence of a TAZ or TAZ-like polypeptide. The term also includes single-stranded or double-stranded molecules, or those nucleic acids that are the complement of nucleic acids encoding said polypeptide, as would be appreciated by those skilled in the art. Therefore, we include in this term all nucleic acid molecules that encode a TAZ or TAZ-like polypeptide as

defined above, including homologues, orthologues and paralogues as identified from the *in silico* screening and database mining methods discussed above.

A preferred embodiment of the first aspect of the invention is wherein the non-human animal has a reduced amount of functional native TAZ polypeptide or a reduced amount of nucleic acid encoding said polypeptide. By "native" we mean that TAZ polypeptide or nucleic acid is encoded by a gene native to the animal.

An alternative embodiment of the first aspect of the invention is wherein the non-human animal has a reduced amount of functional native TAZ-like polypeptide, preferably YAP polypeptide, or a reduced amount of nucleic acid encoding said polypeptide. By "native" we mean that YAP polypeptide or nucleic acid is encoded by a gene native to the animal.

A further alternative embodiment of the first aspect of the invention is wherein the non-human animal has a reduced amount of functional native TAZ and a reduced amount of functional native YAP polypeptide or a reduced amount of nucleic acid encoding said polypeptides.

A further alternative embodiment of the first aspect of the invention is wherein the non-human animal has no functional TAZ polypeptide or no nucleic acid encoding said polypeptide.

A second aspect of the invention provides a method for generating a non-human animal which develops PKD comprising reducing the amount of functional TAZ polypeptide and/or TAZ-like polypeptide, or reducing the amount of nucleic acid encoding said polypeptide.

Non-human animals having a reduced amount of functional TAZ polypeptide and/or TAZ-like polypeptide or a nucleic acid encoding said polypeptide, can be used as disease models in which to study PKD progression and to aid in identifying agents which may be useful as a therapeutic entity.

There are a number of different methods that can be employed to generate a non-human animal according to the first aspect of the invention. These will be discussed in turn below. Preferred methods include those in which the gene encoding the said polypeptide is altered or removed so as to produce little or none of said polypeptide. Other methods include inhibiting the transcription of the said gene or preventing any mRNA encoded by said gene from being translated.

"Homologous recombination" is a technique well known to those skilled in the art. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Pat. No. 5,557,032, incorporated herein by reference. Hence this aspect of the invention includes wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by mutated one or more gene(s) encoding TAZ and/or TAZ-like polypeptide by homologous recombination. As a result the animal will no longer be able to synthesise TAZ and/or TAZ-like polypeptide, i.e. there will be a reduction in the amount of this polypeptide(s). This can also lead to a reduction in the amount of nucleic acid encoding said polypeptide. An example of the use of this method of the invention is provided in the accompanying examples.

FLP/FRT and CRE/LOX recombination systems can also be used to mutate one or more gene(s) encoding TAZ and/or TAZ-like polypeptide, as would be appreciated by a person skilled in the art.

Homologous recombination can be used to modify specific regions of a gene(s) encoding TAZ and/or TAZ-like polypeptide. For example, an introduced TAZ gene lacking certain protein motifs, for example the WW or PDZ motif, may replace a native gene encoding TAZ. Such a method that would generate a non-human animal that has reduced levels of functional TAZ and/or TAZ-like polypeptide is also included in this aspect of the invention.

Homologous recombination can be used to generate a non-human animal in which the amount of functional TAZ polypeptide and/or TAZ-like polypeptide is reduced only in certain cell types, i.e. tissue- or cell-specific knockout, for example a kidney-specific knockout. This can be achieved by introducing a gene encoding a TAZ polypeptide and/or TAZ-like polypeptide which is only expressed in certain cells or tissues using a cell- or tissue-specific promoter element, for example a promoter element not expressed in the kidney or using CRE/LOX recombination system.

"Insertional mutagenesis" is also a term well known to those skilled in the art. Examples of such mutagenesis include transposon-tagging, homing endonuclease genes (HEGs). In such methods a region of DNA is introduced into a gene such that the controlling or coding region of the gene is disrupted. Practical methods of using insertional mutagenesis in animals are well known to those skilled in the art. Hence a further embodiment of this aspect of the invention is wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by mutated one or more gene(s) encoding TAZ and/or TAZ-like polypeptide by insertional

mutagenesis. As a result the animal will no longer be able to synthesise TAZ and/or TAZ-like polypeptide, i.e. there will be a reduction in the amount of this polypeptide(s). This can also lead to a reduction in the amount of nucleic acid encoding said polypeptide.

Chemical or physical mutagenesis can also be used in the method of this aspect of the invention. Here, a gene is mutated by exposing the genome to a chemical mutagen, for example ethyl methylsulphate (EMS) or ethyl mitrosourea (ENU), or a physical mutagen, for example X-rays. Such agents act to alter the polynucleotide sequence of a gene or, in the case of some physical mutagens, can rearrange the order of sequences in a gene. Practical methods of using chemical or physical mutagenesis in animals are well known to those skilled in the art. Hence a further embodiment of this aspect of the invention is wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by mutated one or more gene(s) encoding TAZ and/or TAZ-like polypeptide by chemical or physical mutagenesis. As a result the animal will no longer be able to synthesise TAZ and/or TAZ-like polypeptide, i.e. there will be a reduction in the amount of this polypeptide(s). This can also lead to a reduction in the amount of nucleic acid encoding said polypeptide.

Homologous recombination, insertional mutagenesis and chemical or physical mutagenesis can be used in the method of the second aspect of the invention to generate a non-human animal which is heterozygous for the target gene, e.g. $TAZ^{+/-}$. Such animals may be of particular use if the method of the second aspect of the invention generates a non-human animal having too severe a phenotype. As set out above, $TAZ^{-/-}$ mice develop PKD and can be used as disease models in which to study PKD progression and to aid in identifying agents which may be useful as a therapeutic entity.

Antisense oligonucleotides are single-stranded nucleic acids, which can specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA duplex is formed. These nucleic acids are often termed "antisense" because they are complementary to the sense or coding strand of the gene. Recently, formation of a triple helix has proven possible where the oligonucleotide is bound to a DNA duplex. It was found that oligonucleotides could recognise sequences in the major groove of the DNA double helix. A triple helix was formed thereby. This suggests that it is possible to synthesise a sequence-specific molecules which specifically bind double-stranded DNA *via* recognition of major groove hydrogen binding sites.

By binding to the target nucleic acid, the above oligonucleotides can inhibit the function of the target nucleic acid. This could, for example, be a result of blocking the transcription, processing, poly(A)addition, replication, translation, or promoting inhibitory mechanisms of the cells, such as promoting RNA degradations.

Antisense oligonucleotides are prepared in the laboratory and then introduced into cells, for example by microinjection or uptake from the cell culture medium into the cells, or they are expressed in cells after transfection with plasmids or retroviruses or other vectors carrying an antisense gene. Antisense oligonucleotides were first discovered to inhibit viral replication or expression in cell culture for Rous sarcoma virus, vesicular stomatitis virus, herpes simplex virus type 1, simian virus and influenza virus. Since then, inhibition of mRNA translation by antisense oligonucleotides has been studied extensively in cell-free systems including rabbit reticulocyte lysates and wheat germ extracts. Inhibition of viral function by antisense oligonucleotides has been demonstrated *in vitro* using

oligonucleotides which were complementary to the AIDS HIV retrovirus RNA (Goodchild, J. 1988 "Inhibition of Human Immunodeficiency Virus Replication by Antisense Oligodeoxynucleotides", *Proc. Natl. Acad. Sci. (USA)* **85**(15), 5507-11). The Goodchild study showed that oligonucleotides that were most effective were complementary to the poly(A) signal; also effective were those targeted at the 5N end of the RNA, particularly the cap and 5N untranslated region, next to the primer binding site and at the primer binding site. The cap, 5N untranslated region, and poly(A) signal lie within the sequence repeated at the ends of retrovirus RNA (R region) and the oligonucleotides complementary to these may bind twice to the RNA.

Typically, antisense oligonucleotides are 15 to 35 bases in length. For example, 20-mer oligonucleotides have been shown to inhibit the expression of the epidermal growth factor receptor mRNA (Witters *et al, Breast Cancer Res Treat* 53:41-50 (1999)) and 25-mer oligonucleotides have been shown to decrease the expression of adrenocorticotropic hormone by greater than 90% (Frankel *et al, J Neurosurg* 91:261-7 (1999)). However, it is appreciated that it may be desirable to use oligonucleotides with lengths outside this range, for example 10, 11, 12, 13, or 14 bases, or 36, 37, 38, 39 or 40 bases.

By "antisense" we also include all methods of RNA interference, which are regarded for the purposes of this invention as a type of antisense technology.

Therefore, a further embodiment of this aspect of the invention is wherein the amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide is reduced using antisense. As a result the animal will no longer be able to synthesise TAZ and/or TAZ-like polypeptide, i.e. there will be a

reduction in the amount of said polypeptide(s). This can also lead to a reduction in the amount of nucleic acid encoding said polypeptide.

Antisense can also be used to generate a non-human animal in which the amount of functional TAZ polypeptide and/or TAZ-like polypeptide is reduced only in certain cell types, i.e. a tissue- or cell-specific knockout, for example a kidney-specific knockout. This can be achieved by preparing the oligonucleotides in the laboratory and then introducing them into specific cells, for example by microinjection or implanting them using liposomes, microcapsules or implantable devices, for example into the kidney. Alternatively, it is possible to generate tissue- or cell-specific knockouts by expressing antisense oligonucleotides in specific cells after transfection with plasmids or retroviruses or other vectors carrying an antisense gene which is only expressed in specific cells or tissues using a cell- or tissue-specific promoter element, for example a kidney cell- specific promoter element, or a regulatable promoter, as would be appreciated by a person skilled in the art.

It will be appreciated that antisense agents also include larger molecules which bind to mRNA or genes encoding TAZ polypeptide and/or TAZ-like polypeptide and substantially prevent expression of said mRNA or genes. Thus, expression of such an antisense molecule which is substantially complementary to nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide and is envisaged as part of the invention.

The said larger molecules may be expressed from any suitable genetic construct as is described below and delivered to the patient. Typically, the genetic construct which expresses the antisense molecule comprises at least a portion of the cDNA or gene encoding the TAZ polypeptide and/or TAZ-like

polypeptide, operatively linked to a promoter which can express the antisense molecule in the cell.

In a further embodiment of this aspect of the invention is wherein the amount of nucleic acid encoding TAZ polypeptide and/or a TAZ-like polypeptide is reduced by supplying the animal with a ribozyme capable of cleaving RNA or DNA encoding TAZ polypeptide and/or TAZ-like polypeptide. A gene expressing said ribozyme may be administered in substantially the same and using substantially the same vehicles as for antisense molecules.

Ribozymes which may be encoded in the genomes of the viruses or virus-like particles herein disclosed are described in Cech and Herschlag "Site-specific cleavage of single stranded DNA" US 5,180,818; Altman *et al* "Cleavage of targeted RNA by RNAse P" US 5,168,053; Cantin *et al* "Ribozyme cleavage of HIV-1 RNA" US 5,149,796; Cech *et al* "RNA ribozyme restriction endoribonucleases and methods", US 5,116,742; Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endonucleases and methods", US 5,093,246; and Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endoribonucleases and methods; cleaves single-stranded RNA at specific site by transesterification", US 4,987,071, all incorporated herein by reference.

It will be appreciated that it may be desirable that the antisense molecule or ribozyme is expressed from a cell-specific promoter element, or a regulatable promoter.

The genetic constructs of the invention can be prepared using methods well known in the art.

A further method for reducing the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide in a non-human animal is by supplying the animal with one or more agents that act as an antagonist of TAZ and/or a TAZ-like polypeptide.

The term "antagonist" is well known to those skilled in the art. By "antagonist" we include any agent that acts to reduce the level of functional TAZ polypeptide and/or a TAZ-like polypeptide. An example of such an antagonist is an antibody, or antibody fragment, that binds to one of the said polypeptides such that the polypeptide cannot effect its normal function. The antagonist may also alter the sub-cellular localisation of TAZ or TAZ-like polypeptide. In this way, the level of functional polypeptide is reduced. The antagonist may act to affect the function of the WW or PDZ motifs present in TAZ polypeptide and/or a TAZ-like polypeptide thus modifying the polypeptide's capacity to function as a transcriptional coactivator.

The antagonist may be used to generate a tissue- or cell-specific knockout, for example a kidney-specific knockout, of TAZ polypeptide and/or a TAZ-like polypeptide function. For example, where the antagonist is an antibody, or antibody fragment, that binds to one of the said polypeptides such that the polypeptide cannot effect a normal function or alter its sub-cellular localisation, then a gene encoding said antagonist may be regulated using a tissue- or cell- specific promoter, for example a kidney-specific promoter.

Further methods of use in this aspect of the invention include wherein the amount of nucleic acid encoding TAZ polypeptide and/or a TAZ-like polypeptide is reduced by modifying the chromatin structure at or adjacent to gene(s) encoding a TAZ polypeptide and/or a TAZ-like polypeptide. This may be achieved using, for example, targeted DNA methylation. Such methods are known to those skilled in the art.

Further methods of use in this aspect of the invention include wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by modifying the function of an upstream regulator of TAZ polypeptide and/or a TAZ-like polypeptide. For example, phosphorylation by Ser/Thr kinase(s) is required for TAZ to interact with 14-3-3 proteins (Kanai et al, *supra*). Therefore, modifying the function of an endogenous Ser/Thr kinase, preferably a kidney-specific kinase, would lead to a reduction in the amount of functional TAZ polypeptide and/or TAZ-like polypeptide.

Further methods of use in this aspect of the invention include wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by supplying the animal with a dominant inactive form of a TAZ and/or a TAZ-like polypeptide. For example a polypeptide may be modified so as to generate a dominant inactive form of a TAZ polypeptide or a TAZ-like polypeptide that can bind to the same binding sites as TAZ or TAZ-like polypeptides but cannot effect the same function as TAZ polypeptide and/or a TAZ-like polypeptide. Alternatively the dominant inactive form may be mislocalised within the cell. Hence overexpression of a dominant inactive form of a TAZ polypeptide and/or a TAZ-like polypeptide in a non-human animal may act to block the function of the native TAZ polypeptide or TAZ-like polypeptide.

A third aspect of the invention provides a method for generating a non-human animal which develops PKD comprising:

i) identifying a polypeptide regulated by TAZ or a TAZ-like polypeptide, or a nucleic acid encoding said polypeptide;

ii) generating a non-human animal which has a reduced amount of the polypeptide and/or nucleic acid identified in step i); and optionally,

iii) selecting a non-human animal generated by step ii) which develops PKD.

The first step of the method of the third aspect of the invention identifies a polypeptide regulated by TAZ or a TAZ-like polypeptide, or a nucleic acid encoding said polypeptide.

As mentioned above, TAZ acts as a coactivator to regulate the function of further polypeptides, for example transcription factors. Hence it is likely that the PKD phenotype of $TAZ^{-/-}$ results from TAZ failing to activate the function of a polypeptide or nucleic acid regulated by TAZ. For example, Kanai et al, *supra* disclose that TAZ functions as a transcriptional coactivator of Runx transcription factors. The *Runx2* gene (also known as *Cbfla*) is expressed in the kidney (see http://www.rzpd.de/cgi-bin/cards/carddisp?RUNX2&search=runx2&suff=txt) and therefore may in part mediate the PKD phenotype of $TAZ^{-/-}$ mice. TAZ also interacts with thyroid transcription factor 1 (TTF1, also called Nkx2.1). Kanai et al, *supra* speculate that transcription factors having a PPXY motif, for example c-Jun, AP-2, C/EBPα, NF-E2, KROZ-20, KROX-24, Oct-4, MEF2B and the p53 homologue p73, may be regulated by TAZ. Therefore the term "polypeptide regulated by TAZ or TAZ-like polypeptide" includes those mentioned above.

TAZ and TAZ-like polypeptides can regulate polypeptides in two ways.

Firstly, TAZ and TAZ-like polypeptides can directly act to regulate the function of further polypeptides, for example Cbf1a and TTF1 as mentioned above. Further examples of polypeptides included with the scope of the term "polypeptide regulated by TAZ or TAZ-like polypeptide" may be found by, for example, a yeast two-hybrid library screen using a TAZ or TAZ-like polypeptide or a fragment or variant thereof, as a bait, as would be appreciated by those skilled in the art.

Secondly, TAZ and TAZ-like polypeptides can indirectly act to regulate the function of further polypeptides. For example, TAZ can interact with TTF1 to regulate the expression of surfactant protein C (Park et al, *supra*). Further examples of polypeptides included with the scope of the term "polypeptide regulated by TAZ or TAZ-like polypeptide" may be found by, for example, conducting a proteomic or transcriptional microarray analysis of normal tissue and tissue having a reduced amount of functional TAZ or TAZ-like polypeptide, and identifying those polypeptides or transcripts which are present in different amounts, as would be appreciated by those skilled in the art. Methods to identify such polypeptides are provided in example 2.

Further methods of identifying polypeptides regulated by TAZ or TAZ-like polypeptide include "pull down" assays in which the TAZ polypeptide is fused to a GST tag; the tagged polypeptide is bound to glutathione beads; the tagged polypeptide is then incubated with a sample of further polypeptides; any polypeptides that interact with TAZ or TAZ-like polypeptide are then isolated from the beads and identified using mass spectrometry. Alternatively, the "pull down" assay can use an antibody to isolate any polypeptides that interact with TAZ or TAZ-like polypeptide by precipitating the TAZ/polypeptide complex.

A further method of identifying polypeptides regulated by TAZ or TAZ-like polypeptide is wherein nucleic acid encoding TAZ is transcribed and translated in the presence of one or more cDNAs that encode further polypeptides. By assaying the amount or function of TAZ it is possible to determine whether the further polypeptide can be considered to interact with TAZ.

The second step of the method of the third aspect of the invention generates a non-human animal which has a reduced amount of the polypeptide and/or nucleic acid identified in the first step.

Methods of generating an animal having a reduced amount of a polypeptide, or a nucleic acid encoding said polypeptide, are discussed above in relation to the second aspect of the invention. For example, it is possible to use homologous recombination, insertional mutagenesis or chemical or physical mutagens to disrupt the nucleic acid sequence encoding the polypeptide identified in the first step of this aspect of the invention. Further methods included in this aspect of the invention include antisense, supplying the animal with an antagonist of the identified polypeptide, modifying the chromatin structure at or adjacent to the gene encoding the identified polypeptide, modifying the function of an upstream regulator of the identified polypeptide, or supplying a non-human animal with a dominant inactive form of the identified polypeptide.

A final optional step in the third method of the third aspect of the invention involves selecting a non-human animal generated by step ii) which develops PKD.

PKD is commonly associated with multiple cysts locates in the kidney. Hence one method of selecting whether a non-human animal generated by

step ii) develops PKD is to examine the kidney of a test animal and compare the amount, location or physiology of cysts to a reference animal, i.e. an animal which does not have a reduced amount of the polypeptide and/or nucleic acid identified in step i). PKD progression can also be measured by ultrasound analysis or the use of a blood test that measures creatinine, urea and nitrogen levels, all of which are characteristically high in animals with PKD.

A fourth aspect of the invention provides a non-human animal obtained or obtainable from the method of the third aspect of the invention.

A fifth aspect of the invention provides a method of screening for compounds of use in preventing or treating PKD wherein a non-human animal is administered a test compound and the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide function, or the amount of nucleic acid encoding said polypeptide, is assessed.

An embodiment of the fifth aspect of the invention is wherein the animal is a genetically modified non-human animal according to the first or fourth aspects of the invention.

A sixth aspect of the invention provides a method of screening for compounds of use in preventing or treating PKD wherein a cell having TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ function is treated with a test compound and the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like

polypeptide, or the amount of nucleic acid encoding said polypeptide, is assessed.

The fifth and sixth aspects of the invention relate to screening methods for drugs or lead compounds. This will be further discussed below. The test compounds used in the methods of these aspects of the invention may increase or decrease the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide function, or the amount of nucleic acid encoding said polypeptide. An increase in such functions or levels is generally preferred as such test compounds may be of use for preventing or treating PKD.

However, the methods of the fifth and sixth aspects of the invention also include the purpose of identifying compounds that decrease the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide function, or the amount of nucleic acid encoding said polypeptide. Such compounds may be of use for developing a model animal system having accelerated PKD or a cell-based assay having suppressed functions or levels of TAZ polypeptide and/or a ~~TAZ~~-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide; such model animals or cell-based assays can be used to determine whether further test compounds, or other external stimuli, may be of use for preventing or treating PKD.

A seventh aspect of the invention provides a method of identifying a compound of use in preventing or treating PKD comprising:

i) administering a test compound to a non-human animal;

ii) assessing the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of a nucleic acid encoding said polypeptide; and,

iii) selecting any compound that increases the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or increases the amount of nucleic acid encoding said polypeptide.

An eighth aspect of the invention provides a method of identifying a compound of use in preventing or treating PKD comprising:

i) administering a test compound to TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide;

ii) assessing the effect of the test compound on the function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide function, and,

iii) selecting any compound that increases the function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide.

A ninth aspect of the invention provides a method of identifying a compound of use in preventing or treating PKD comprising:

i) administering a test compound to cell comprising TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide;

ii) assessing the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of a nucleic acid encoding said polypeptide; and,

iii) selecting any compound that increases the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of a nucleic acid encoding said polypeptide.

The methods of the fifth, sixth, seventh, eighth and ninth aspects of the invention relate to screening methods for drugs or lead compounds.

The term "polypeptide regulated by TAZ or TAZ-like polypeptide" includes those polypeptide mentioned above in relation to the third aspect of the invention.

As would be understood by the skilled person, in the methods of the fifth, sixth, seventh, eighth and ninth aspects of the invention the terms "TAZ polypeptide", "TAZ-like polypeptide" and "a polypeptide regulated by TAZ or a TAZ-like polypeptide" also includes fragments and variants of these polypeptides.

A "fragment" of the TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or TAZ-like polypeptide is one which may retain one or more activities of the full length polypeptide. For example,

TAZ has a number of different protein motifs, e.g. the WW domain and the PDZ-binding motif. Hence a fragment comprising one or more of such domains isolated from the said polypeptide(s) can also be used in the fifth, sixth, seventh, eighth and ninth aspects of the invention.

A "variation" of the fragment of the polypeptide is one which may usable to to prepare antibodies which specifically bind to the protein. Such a variant may be encoded by a gene in which different codons can be substituted which code for the same amino acid(s) as the original codons. Alternatively, the substitute codons may code for a different amino acid that will not affect the function or immunogenicity of the protein or which may improve its function or immunogenicity. For example, site-directed mutagenesis or other techniques can be employed to create single or multiple mutations, such as replacements, insertions, deletions, and transpositions, as described in Botstein and Shortle, "Strategies and Applications of *In Vitro* Mutagenesis," *Science*, **229:** 193-1210 (1985), which is incorporated herein by reference.

It will be appreciated that in the methods fifth, sixth, seventh, eighth and ninth aspects of the invention, which may be drug screening methods, a term well known to those skilled in the art, the compound may be a drug-like compound or lead compound for the development of a drug-like compound.

The term "drug-like compound" is well known to those skilled in the art, and may include the meaning of a compound that has characteristics that may make it suitable for use in medicine, for example as the active ingredient in a medicament. Thus, for example, a drug-like compound may be a molecule that may be synthesised by the techniques of organic chemistry, less preferably by techniques of molecular biology or biochemistry, and is preferably a small molecule, which may be of less than

5000 daltons and which may be water-soluble. A drug-like compound may additionally exhibit features of selective interaction with a particular protein or proteins and be bioavailable and/or able to penetrate target cellular membranes, but it will be appreciated that these features are not essential.

The term "lead compound" is similarly well known to those skilled in the art, and may include the meaning that the compound, whilst not itself suitable for use as a drug (for example because it is only weakly potent against its intended target, non-selective in its action, unstable, poorly soluble, difficult to synthesise or has poor bioavailability) may provide a starting-point for the design of other compounds that may have more desirable characteristics.

The methods of the fifth, sixth, seventh, eighth and ninth aspects of the invention include a step of assessing the effect of a test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide, and the fifth, sixth, seventh and ninth aspects of the invention also include a step of assessing the effect of a test compound on the amount of nucleic acid encoding said polypeptide.

In common with all these methods is the need for a "reference sample", i.e. a sample of protein or nucleic acid taken from an animal or a cell or a test reaction which has not been exposed to the test compound. By comparing the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide, in a sample of protein or nucleic acid taken from an animal or a cell or a test reaction which has not been exposed to the test compound, to the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated

by TAZ or TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide, in a sample of protein or nucleic acid taken from an animal or a cell or a test reaction which has been exposed to the test compound it is possible to determine the effect of the test compound on the amount of function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide. This will be either an elevation or reduction in the amount or function of the polypeptide or the amount of nucleic acid.

The step of assessing the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide, may be performed using a number of different methods.

Firstly, the effect of the test compound in the fifth, sixth, seventh and ninth aspects of the invention can be determined by quantifying the amount of nucleic acid, preferably mRNA, encoding the TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide.

Levels of mRNA encoding the TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide may be assayed using the RT-PCR method described in Makino et al, Technique 2:295-301 (1990). By this method, the radioactivities of the "amplicons" in the polyacrylamide gel bands are linearly related to the initial concentration of the target mRNA. Briefly, this method involves adding total RNA isolated from a biological sample in a reaction mixture containing a RT primer and appropriate buffer. After incubating for primer annealing, the mixture can be supplemented with a RT buffer, dNTPs, DTT,

RNase inhibitor and reverse transcriptase. After incubation to achieve reverse transcription of the RNA, the RT products are then subject to PCR using labeled primers. Alternatively, rather than labeling the primers, a labeled dNTP can be included in the PCR reaction mixture. PCR amplification can be performed in a DNA thermal cycler according to conventional techniques. After a suitable number of rounds to achieve amplification, the PCR reaction mixture is electrophoresed on a polyacrylamide gel. After drying the gel, the radioactivity of the appropriate bands (corresponding to the mRNA encoding the TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide) is quantified using an imaging analyzer. RT and PCR reaction ingredients and conditions, reagent and gel concentrations, and labeling methods are well known in the art. Variations on the RT-PCR method will be apparent to the skilled artisan. Any set of oligonucleotide primers which will amplify reverse transcribed target mRNA can be used and can be designed as will be well known to those skilled in the art.

Levels of mRNA encoding the TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide can also be assayed using northern blotting, a method well known to those skilled in the art and described further in Sambrook *et al.*, Molecular Cloning. A laboratory manual. 1989. Cold Spring Harbour publications.

Further methods which may be of use in measuring mRNA levels include *in situ* hybridisation (In Situ Hybridization Protocols. Methods in Molecular Biology Volume 33. Edited by K H A Choo. 1994, Humana Press Inc (Totowa, NJ, USA) pp 480p and In Situ Hybridization: A Practical Approach. Edited by D G Wilkinson. 1992, Oxford University Press,

Oxford, pp 163), *in situ* amplification, nuclease protection, probe arrays, and amplification based systems.

A further method of assessing the effect of the test compound on TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide in the fifth, sixth, seventh or ninth aspects of the invention is to quantify the amount of said polypeptide.

Assaying the amount of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide in a biological sample can occur using any art-known method. Preferred for assaying TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein levels in a biological sample are antibody-based techniques. For example, TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein expression in tissues can be studied with classical immunohistological methods. In these, the specific recognition is provided by the primary antibody (polygonal or monoclonal) but the secondary detection system can utilize fluorescent, enzyme, or other conjugated secondary antibodies. As a result, an immunohistological staining of tissue section for pathological examination is obtained. Tissues can also be extracted, e.g., with urea and neutral detergent, for the liberation of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein for Western-blot or dot/slot assay (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987)). In this technique, which is based on the use of cationic solid phases, quantitation of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein can be accomplished using isolated TAZ polypeptide and/or a TAZ-like polypeptide and/or a

polypeptide regulated by TAZ or TAZ-like polypeptide protein as a standard. This technique can also be applied to animal fluids. With these samples, a molar concentration of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein will aid to set standard values of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein content for different animal fluids, like serum, plasma, urine, spinal fluid, etc. The normal appearance of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein amounts can then be set using values from healthy individuals, which can be compared to those obtained from a test subject.

Other antibody-based methods useful for detecting TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). For example, a TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein-specific monoclonal antibody can be used both as an immunoadsorbent and as an enzyme-labeled probe to detect and quantify the TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein. The amount of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein present in the sample can be calculated by reference to the amount present in a standard preparation using a linear regression computer algorithm. Such an ELISA for detecting a tumor antigen is described in Iacobelli et al., Breast Cancer Research and Treatment 11: 19-30 (1988). In another ELISA assay, two distinct specific monoclonal antibodies can be used to detect TAZ polypeptide and/or a TAZ-like

polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein in a body fluid. In this assay, one of the antibodies is used as the immunoadsorbent and the other as the enzyme-labeled probe.

In addition to assaying TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein levels in a biological sample obtained from an animal or a cell, TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein can also be detected *in vivo* by imaging. Antibody labels or markers for in vivo imaging of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or caesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide protein-specific antibodies for use in the screening methods of the present invention can be raised against the intact TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ protein or TAZ-like polypeptide or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al, J. Nucl. Med. 24:316-325 (1983)). Thus, these fragments are preferred.

A further method of assessing the effect of the test compound in the fifth, sixth, seventh, eighth and ninth aspects of the invention is to assess the effect of the test compound on the function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide.

As discussed above, TAZ acts as a transcriptional coactivator. Hence determining any change in the capacity of TAZ to act as a transcriptional coactivator can be used to assess the effect of a test compound on TAZ function.

An assay of TAZ function is presented in Kanai et al, *supra*. Here TAZ is fused to the minimum GAL4 DNA-binding domain and the effect of TAZ-GAL4 DBD on regulating the transcription of a reporter gene containing a GAL4 DNA-binding site is assessed. Such an assay may be of use in the screening methods of the invention. For example, in the sixth aspect of the invention the test compound may be administered to a TAZ polypeptide comprising a GAL4 DBD and the effect of the test compound on the capacity of the TAZ polypeptide to activate reporter gene expression assessed.

A reporter protein may be one whose activity may easily be assayed, for example β-galactosidase, chloramphenicol acetyltransferase or luciferase (see, for example, Tan *et al* (1996)). In a further example, the reporter gene may be fatal to the cells, or alternatively may allow cells to survive under otherwise fatal conditions. Cell survival can then be measured, for example using colorimetric assays for mitochondrial activity, such as reduction of WST-1 (Boehringer). WST-1 is a formosan dye that undergoes a change in absorbance on receiving electrons *via* succinate dehydrogenase.

TAZ polypeptide can also interact with further polypeptides to regulate gene expression. For example, in Cui et al *supra* an assay of TAZ function is disclosed in which TAZ is cotransformed into cells with a reporter gene construct (in this case luciferase) and a polypeptide which TAZ binds to effect transactication (in this case Cbfa1); the level of reporter gene activity reflects TAZ function. Such an assay may be of use in the screening methods of the invention. For example, in the eighth aspect of the invention the test compound may be administered to a TAZ polypeptide and the effect of the test compound on the capacity of the TAZ polypeptide to activate reporter gene expression through Cbfa1 assessed.

Similar assays of use in the screening methods of the invention are also disclosed in Park et al *supra*.

TAZ and the TAZ-like polypeptide YAP participate in specific protein-protein interactions (Kanai et al, *supra*). Therefore, one assay of TAZ and TAZ-like function is to assess the effect of the test compound on the capacity of TAZ and TAZ-like polypeptide to participate in such interactions. Kanai et al, *supra* present assays in which TAZ and YAP bind to NHERF-2. Zaidi et al (2004) EMBO J 23, 790-799 present data showing that YAP interacts with Runx2. Such assays may be of use in the screening

methods of the invention. For example, in the eighth aspect of the invention the test compound may be administered to a TAZ or TAZ-like polypeptide and the effect of the test compound on the capacity of the polypeptides to interact with NHERF-2 assessed. Further possible assays would be apparent to those skilled in the art.

A tenth aspect of the invention provides a method of identifying a compound of use in modulating PKD progression comprising:

i) administering a test compound to a non-human animal which develops PKD according to the first or fourth aspects of the invention;

ii) assessing the effect of the test compound on PKD; and,

iii) selecting any compound that modulates PKD progression in the animal.

The tenth aspect of the invention is also a screening method for drugs or lead compounds.

The methods of the tenth aspect of the invention may include a further step of assessing the effect of the test compound on PKD. Hence there is the need for a "reference sample", i.e. an animal which has not been exposed to the test compound. By comparing PKD in a test animal to an animal exposed to the test compound it may be possible to determine the effect of the test compound on PKD progression.

PKD is commonly associated with multiple cysts locates in the kidney. Hence one method to assess the effect of the test compound on PKD is to

examine the kidney of a test animal and compare the amount, location or physiology of cysts to a reference animal. PKD progression can also be measured by ultrasound analysis or the use of a blood test that measures creatinine, urea and nitrogen levels, all of which are characteristically high in PKD patients.

The term "modulates PKD progression" includes increasing or reducing PKD progression. A preferred embodiment of the tenth aspect of the invention is wherein the compound selected in step iii) reduces PKD progression.

However, the tenth aspect of the invention may also be used to identify test compounds that increase PKD progression. Such compounds may be of use for developing a model animal system having accelerated PKD; this can be used to determine whether further test compounds, or other external stimuli, may be of use for preventing or treating PKD.

The screening methods of the invention can be used in "library screening" methods, a term well known to those skilled in the art. Thus, for example, the methods of the invention may be used to detect (and optionally identify) a test compound capable of modifying TAZ, TAZ-like or a polypeptide regulated by TAZ or TAZ-like polypeptide, or PKD progression. Aliquots of a library may be tested for the ability to give the required result.

Compounds that have been identified from the screening methods of the invention as of use in preventing or treating PKD can subsequently be further studied to determine their effect on, for example, the quantity of cysts, or to measure the effect of the identified compounds on creatinine, urea and nitrogen levels, as discussed above.

A further embodiment of the method of the fifth, sixth, seventh, eighth, ninth and tenth aspects of the invention is wherein compound for use in preventing or treating PKD is formulated into a pharmaceutically acceptable composition.

An eleventh aspect of the invention provides a method of making a compound useful in preventing or treating PKD comprising synthesising a compound identified or selected by any one of the method of the fifth, sixth, seventh, eighth, ninth and tenth aspects of the invention.

A twelfth aspect of the invention provides a pharmaceutical composition comprising the method of the fifth, sixth, seventh, eighth, ninth and tenth aspects of the invention and the step of mixing the compound of use in preventing or treating PKD with a pharmaceutically acceptable carrier.

Preferably, the formulation is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

The compounds will normally be administered orally or by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

In human therapy, the compounds can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The compounds may also be administered *via* intracavernosal injection.

The compounds can also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

A thirteenth aspect of the invention provides a method for assessing a patient's risk of developing PKD or progression of PKD, comprising the steps of:

(i) obtaining a sample containing nucleic acid and/or protein from the patient; and,

(ii) determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or TAZ-like polypeptide, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or TAZ-like polypeptide,

and/or determining the patient's genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or TAZ-like polypeptide.

This method may be useful in the diagnosis of PKD or as a basis of genetic counselling.

Methods of determining the amount and function of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or TAZ-like polypeptide are presented above in relation to the screening methods of the invention.

Methods of determining the location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide include *in situ* immuno-histochemistry analysis using an antibody that recognises said polypeptide, as would be appreciated by a person skilled in the art; or cell fractionation followed by an immunoassay for the presence of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, again as would be appreciated by a person skilled in the art.

Methods of determining the amount of nucleic acid encoding TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide include northern blotting and RT-PCT as presented above in relation to the screening methods of the invention. Furthermore, it is possible that genomic rearrangements can lead to an increase in the copy number of gene(s) encoding TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, i.e. nucleic acids encoding said polypeptide. Methods of determining gene copy number include Southern blotting (essentially as performed as set out in Sambrook *et al*

(1989). Molecular cloning, a laboratory manual, 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, New York) or quantitative PCR.

Methods of determining the patient's genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide include determining whether the patient has one or more mutation(s) in the gene, or complete absence of the gene(s) encoding TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, leading to reduced or nil expression of the polypeptide(s) or expression of functionally inactive versions of the polypeptide(s). By "gene" we include the coding region and the controlling region, e.g. the promoter, of the gene. Such genetic assay methods include the standard techniques of restriction fragment length polymorphism assays and PCR-based assays.

The assay may involve any suitable method for identifying such polymorphisms, such as: sequencing of the DNA at one or more of the relevant positions; differential hybridisation of an oligonucleotide probe designed to hybridise at the relevant positions of either the wild-type or mutant sequence; denaturing gel electrophoresis following digestion with an appropriate restriction enzyme, preferably following amplification of the relevant DNA regions; S1 nuclease sequence analysis; non-denaturing gel electrophoresis, preferably following amplification of the relevant DNA regions; conventional RFLP (restriction fragment length polymorphism) assays; selective DNA amplification using oligonucleotides which are matched for the wild-type sequence and unmatched for the mutant sequence or *vice versa*; or the selective introduction of a restriction site using a PCR (or similar) primer matched for the wild-type or mutant genotype, followed by a restriction digest. The assay may be indirect, ie capable of detecting a mutation at another position or gene which is known to be linked to one or more of the mutant positions. The probes and primers may be fragments of

DNA isolated from nature or may be synthetic. The methods used to determine genotype(s) are well known to those skilled in the art.

An embodiment of the thirteenth aspect of the invention is wherein if the sample has a reduced amount and/or function and/or an abnormal location of a TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, or if the sample has a reduced amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, or if the sample has a deleterious mutation in one or more gene(s) encoding TAZ and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or TAZ-like polypeptide, then the patient is considered to be at risk of developing PKD.

For example, in mice TAZ polypeptide is normally distributed between the cytoplasm and the nucleus, with some peptide being associated at the plasma membrane (Kanai et al, *supra*). TAZ can also accumulate in punctuate nuclear bodies via its C-terminal PDZ-binding motif. In contrast to TAZ, YAP is mainly distributed diffusely within the cytoplasm with only 10% of assayed cells showing a primarily nuclear staining pattern with no punctuate nuclear staining whatsoever. However, mutated TAZ polypeptides lacking the WW- or PDZ-domains have different distribution patterns. Therefore, assessing TAZ location may provide an assay as to the capacity of TAZ to function correctly in the patient and so provide a measure of the patient's risk of developing PKD.

Suitable samples that may be used in the methods of the thirteenth aspect of the invention include those which contain representative samples of the patient's polypeptide and/or nucleic acid. A preferred embodiment of the

thirteenth aspect of the invention is wherein the sample contains cells from the kidney.

A fouteenth aspect of the invention provides a gene therapy vector comprising a polynucleotide which encodes TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide.

Gene therapy vectors typically allow for the expression of a desired polypeptide(s) in a human cell. The vector may be one which allows for selective expression in the target cell by using promoter sequences which work selectively in the target cell type, for example kidney cell-specific expression.

Gene therapy vectors of the invention may be made by any suitable method. For example, a variety of methods have been developed to operably link DNA to vectors *via* complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to modify the DNA encoding the polypeptide of the invention is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* **239**, 487-491.

In this method the DNA to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

It will be appreciated that the gene expression vector of the invention may readily be made using molecular biological techniques which are well known in the art, such as those described in Sambrook *et al* (1989). Molecular cloning, a laboratory manual, 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, New York.

An embodiment of the fourteenth aspect of the invention is wherein the vector comprises a Moloney Leukaemia Virus (MLV) based retroviral vector or a lentiviral vector or adeno-associated vector (AAV). Such vectors are well known to those skilled in the art and may be constructed using the methods disclosed above in relation to this aspect of the invention.

A fifteenth aspect of the invention provides a pharmaceutical composition comprising a polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or a compound identified from the screening methods of the invention or a compound as defined in relation to the eleventh aspect of the invention or a gene therapy vector as defined in the twelfth aspect of the invention and a pharmaceutically acceptable carrier.

A discussion of pharmaceutically acceptable carriers is provided above in relation to the twelfth aspect of the invention.

A sixteenth aspect of the invention provides a polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or a compound identified from the screening methods of the invention or a compound as defined in relation to the eleventh aspect of the invention or a pharmaceutical composition as defined in relation to the screening methods of the invention or in the twelfth or fifteenth aspects of the invention or a gene therapy vector as defined in the fourteenth aspect of the invention for use in medicine.

A seventeenth aspect of the invention provides the use of a polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by

TAZ or a TAZ-like polypeptide or TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or a compound identified from the screening methods of the invention or a compound as defined in relation to the eleventh aspect of the invention or a pharmaceutical composition as defined in relation to the screening methods of the invention or in the twelfth or fifteenth aspects of the invention or a gene therapy vector as defined in the fourteenth aspect of the invention in the manufacture of a medicament for the treatment of a patient with or at risk of developing PKD.

A eighteenth aspect of the invention provides a method of treating a patient with PKD comprising administering to the patient an effective amount of a polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or a compound identified from the screening methods of the invention or a compound as defined in relation to the eleventh aspect of the invention or a pharmaceutical composition as defined in relation to the screening methods of the invention or in the twelfth or fifteenth aspects of the invention or a gene therapy vector as defined in the fourteenth aspect of the invention.

Means and methods of introducing a genetic construct into a cell in an animal body are known in the art. For example, the polynucleotide of use in the fifteenth, sixteenth, seventeenth and eighteenth aspects of the invention may be introduced into the target cells by any convenient method, for example methods involving adenoviruses.

Targeted retroviruses are also available for use in the invention; for example, sequences conferring specific binding affinities may be engineered into preexisting viral *env* genes (see Miller & Vile (1995) *Faseb*

J. **9**, 190-199 for a review of this and other targeted vectors for gene therapy). The tropism of a retroviral vector can be altered by the incorporation of foreign or hybrid envelope proteins (Battini JL, *et al J. Virol.* **66**: 1468-1475; 1992). This can be achieved by insertion of monoclonal antibodies to mouse ecotropic retroviral particles. Alternatively, any chemical modification such as lactose binding to virus particles can increase the range possible target cells for transduction or confer a predictably altered recognition specificity. Retrovirus particles displaying non-viral polypeptides may be used for specific target cells through the non-viral moiety.

Other methods involve simple delivery of the genetic construct into the cell for expression therein either for a limited time or, following integration into the genome, for a longer time. An example of the latter approach includes (preferably kidney-cell-targeted) liposomes (N≅ssander *et al* (1992) *Cancer Res.* **52**, 646-653).

For the preparation of immuno-liposomes MPB-PE (N-[4-(p-maleimidophenyl)butyryl]-phosphatidylethanolamine) is synthesised according to the method of Martin & Papahadjopoulos (1982) *J. Biol. Chem.* **257**, 286-288. MPB-PE is incorporated into the liposomal bilayers to allow a covalent coupling of the antibody, or fragment thereof, to the liposomal surface. The liposome is conveniently loaded with DNA or other genetic construct for delivery to the target cells, for example, by forming the said liposomes in a solution of the DNA or other genetic construct, followed by sequential extrusion through polycarbonate membrane filters with 0.6 μm and 0.2 μm pore size under nitrogen pressures up to 0.8 MPa. After extrusion, entrapped DNA construct is separated from free DNA construct by ultracentrifugation at 80 000 x g for 45 min. Freshly prepared MPB-PE-liposomes in deoxygenated buffer are mixed with freshly prepared antibody

(or fragment thereof) and the coupling reactions are carried out in a nitrogen atmosphere at 4°C under constant end over end rotation overnight. The immunoliposomes are separated from unconjugated antibodies by ultracentrifugation at 80 000 x g for 45 min. Immunoliposomes may be injected intraperitoneally or directly into the target tissue.

Examples of polynucleotides that encode a TAZ polypeptide or a TAZ-like polypeptide are disclosed above in relation to the first aspect of the invention and are presented in Figures 3 and 4. Further polynucleotides of use in the fifteenth, sixteenth, seventeenth and eighteenth aspects of the invention may be identified according to methods set out in relation to the first aspect of the invention.

The fifteenth, sixteenth, seventeenth and eighteenth aspects of the invention includes TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide.

Polypeptide may be delivered using an injectable sustained-release drug delivery system. These are designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period.

The polypeptide can be administered by a surgically implanted device that releases the drug directly to the required site. For example, Vitrasert releases ganciclovir directly into the eye to treat CMV retinitis. The direct application of this toxic agent to the site of disease achieves effective therapy without the drug's significant systemic side-effects.

Electroporation therapy (EPT) systems can also be employed for the administration of polypeptide. A device which delivers a pulsed electric field to cells increases the permeability of the cell membranes to the drug, resulting in a significant enhancement of intracellular drug delivery.

Polypeptide can be delivered by electroincorporation (EI). EI occurs when small particles of up to 30 microns in diameter on the surface of the skin experience electrical pulses identical or similar to those used in electroporation. In EI, these particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

An alternative method of polypeptide delivery is the ReGel injectable system that is thermo-sensitive. Below body temperature, ReGel is an injectable liquid while at body temperature it immediately forms a gel reservoir that slowly erodes and dissolves into known, safe, biodegradable polymers. The active drug is delivered over time as the biopolymers dissolve.

Polypeptide pharmaceuticals can also be delivered orally. The process employs a natural process for oral uptake of vitamin B_{12} in the body to co-deliver proteins and peptides. By riding the vitamin B_{12} uptake system, the protein or peptide can move through the intestinal wall. Complexes are synthesised between vitamin B_{12} analogues and the drug that retain both significant affinity for intrinsic factor (IF) in the vitamin B_{12} portion of the complex and significant bioactivity of the drug portion of the complex.

Polypeptides can be introduced to cells by "Trojan peptides". These are a class of polypeptides called penetratins which have translocating properties

and are capable of carrying hydrophilic compounds across the plasma membrane. This system allows direct targetting of oligopeptides to the cytoplasm and nucleus, and may be non-cell type specific and highly efficient. See Derossi *et al* (1998), *Trends Cell Biol* **8**, 84-87.

Examples of TAZ or a TAZ-like polypeptide are disclosed above in relation to the first aspect of the invention and are presented in Figures 3 and 4. Further polypeptides of use in the fifteenth, sixteenth, seventeenth and eighteenth aspects of the invention may be identified according to methods set out in relation to the third aspect of the invention.

A nineteenth aspect of the invention provides the use of an agent which is capable of determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide, and/or determining the genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide in the manufacture of a reagent for diagnosing PKD.

Agents that can be used in this aspect of the invention to determine the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide include antibodies or peptide or compounds that can to said polypeptides. Furthermore, as set out above in relation to the screening methods of the invention, agents that may be used to assess the function of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide include reporter genes and regulatable by said polypeptides and coactivation partners for said polypeptides.

Agents that can be used in this aspect of the invention to determine the amount of nucleic acid encoding TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide include primers, oligonucleotides, or other nucleic acid molecules useful in PCR-based methods, northern blotting and *in situ* hybridisation methods set out above in relation to the screening methods of the invention.

Agents that can be used in this aspect of the invention to determine the genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide include primers, oligonucleotides, or other nucleic acid molecules useful in the methods set out above in relation to the thirteenth aspect of the invention.

A twentieth aspect of the invention provides a method of diagnosing PKD comprising the steps of:

(i) obtaining a sample containing nucleic acid and/or protein from a patient; and,

(ii) determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the patient's genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide.

Methods and agents that can be used in step (ii) of this aspect of the invention have been discussed above in relation to the ninteenth aspect of the invention.

A twenty-first aspect of the invention provides a method of assessing the status of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ in a patient comprising the steps of:

(i) obtaining a sample containing nucleic acid and/or protein from a patient; and,

(ii) determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ, and/or determining the patient's genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ.

Methods and agents that can be used in step (ii) of this aspect of the invention have been discussed above in relation to the seventeenth aspect of the invention.

By "assessing the status" we include determining whether the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ, or the amount of nucleic acid encoding TAZ and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ differs in a sample to that of a reference sample from a further patient. Reference samples have been discussed above in relation to the screening methods of the invention. Similarly, we include determining whether the genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ in the sample is different to the normal sequence of the said genes(s). Using the information gained in step (ii) of this method of the invention, a judgement can be made as to the status of TAZ in the patient;

for example, whether the status of the said polypeptide is similar to that which would lead to PKD. Thus, the method may be useful in the diagnosis of PKD or as a basis of genetic counselling.

A twenty-second aspect of the invention provides a kit of parts useful for diagnosing PKD comprising an agent which is capable of use in assessing the amount or function of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide, or assessing the genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide in a sample.

Agents that may be of use in the twenty-second aspect of the invention have been discussed above in relation to the ninteenth aspect of the invention. For example, agents of use in assessing the amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ include a nucleic acid probe that can be used in northern blotting or an oligonucleotide of use in a RT-PCT reaction; agents of use in assessing the amount of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ polypeptide include antibodies that can bind to the said polypeptide; agents of use in assessing the function of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ polypeptide include reporter genes and coactivation partners as discussed above.

A further embodiment of the twenty-second aspect of the invention is a kit of parts further comprising a negative control and/or a positive control.

A further embodiment of the twenty-second aspect of the invention is a kit of parts further comprising means for separating kidney cells from a sample.

The invention will now be described in more detail, for the purposes of illustration only, in the following Examples and Figures.

Figure 1: Targeting of *Taz* locus *Upper*, the genomic fragment of *Taz* gene containing exon-1 and exon-2. *Middle*, targeting vector containing homology fragments of *Taz* locus with inframe insertion of *lacZ* sequence and *loxp* flanked neomycin cassette at the exon2 with deletion of 384 bp. A diphtheria toxin-A gene cassette is located out side of the homology fragments. *Lower*, the targeted *Taz* locus with disrupted exon2 by inframe *lacZ* and *loxp* flanked neomycin cassette insertion. Length of wild type and mutant fragment detected by Southern hybridization with *Sca*I digestion has been shown. Thick lines represent *Taz* genomic sequences; thin lines, pBluescript sequences; black boxes, *Taz* coding exons; black ovals, *loxP* sequences. gray boxes denoted as *lacZ*, *neo* and DT-A indicate *lacZ* cDNA lacking 3'UTR, neomycin-resistant gene with polyadenylation signal driven by *Pgk1* promoter and diphtheria toxin-A fragment gene with MCI promoter, respectively. Primers (p1+p2) are used for the detection of the wild type allele; primers (p3+p4) or (p3+p5) used for the detection of mutant allele in routine genotyping either by conventional or long-range PCR technique. The fragment 'sp' is the probe used for Southern blotting to identify homologous recombination or targeted allele. B, *BamH*I; P, *Pst*I; S, *Sma*I; Sa, *Sal*I; Sc, *Sca*I.

Figure 2: Gross anatomy and histology of kidney from 6 weeks old TAZ$^{+/1}$ and TAZ$^{-/-}$ litter mates. Note the enlarged anemic kidney for the homozygous animals and the numerous cysts located at the corticomedullary junction.

Figure 3: Human and mouse TAZ sequences.

(A): Human TAZ polypeptide sequence; GenBank Accession Number NP_056287.1

(B): Human TAZ polynucleotide sequence; GenBank Accession Number NM_015472.3

(C): Mouse TAZ polypeptide sequence; GenBank Accession Number CAC17733.

(D): Mouse TAZ polynucleotide sequence; GenBank Accession Number AJ299430.

Figure 4: Human and mouse YAP sequences.

(A): Human YAP polypeptide sequence; GenBank Accession Number NP_006097.

(B): Human YAP polynucleotide sequence; GenBank Accession Number NM_006106.

(C): Mouse YAP polypeptide sequence; GenBank Accession Number AAH39125.

(D): Mouse YAP polynucleotide sequence; GenBank Accession Number BC039125.

(E) Chicken YAP polypeptide sequence; GenBank Accession Number NP_990574.

(F) Chicken YAP polynucleotide sequence; GenBank Accession Number NM_205243.

Figure 5: Histological analysis of organs.

Sections through tissues isolated from $TAZ^{+/-}$ and $TAZ^{-/-}$ mice.

Figure 6: LacZ expression at embryonic stage

LacZ expression in embryonic tissues of $TAZ^{-/-}$ mice.

Example 1: A $TAZ^{-/-}$ mouse.

TAZ (transcriptional co-activator with PDZ-binding motif) is a recently identified transcriptional co-activator with high homology to YAP (yes associated protein) (Kanai et al, *supra*). TAZ is highly expressed in heart, lung, liver and kidney and functions as a transcriptional coactivator by binding, via its single WW domain, to PPXY motifs present in targeted transcription factors (Kanai et al, *supra*). Bone-specific transcription factor (Cbfa1/Runx2) (Cui et al, *supra*) and thyroid transcription factor-1 (TTF-1/Nkx-2.1) (Park et al, *supra*) are targets of TAZ identified to date. In addition, TAZ can bind 14-3-3 and PDZ-domain proteins, interactions that may determine its subcellular localization (Kanai et al, *supra*). We inactivated by homolgous recombination the TAZ gene. $TAZ^{-/-}$ mice show a decreased survival rate and surviving animals develop kidney cysts at the corticomedullary border, reminiscent of human nephronophthisis (Riele et al (1992) Proc Natl Acad Sci USA 89, 5128-5132). Thus, TAZ is not required for kidney development *per se* but plays a critical role in kidney physiology, with mutations in the TAZ gene leading to PKD.

Methods and results

Generation *Taz* knock-out mouse

Construction of *Taz-lacZ* knock-in vector:

A 18-20 kb genomic DNA fragment was isolated by screening recombinant mouse DNA library using a non-radioacvtive PCR technique (Amaravadi and King, 1994). An annotated sequence of the isolated genomic fragment had been downloaded (http://www.genome.ucsc.edu/bestlinks.html) and

exon-intron organization and partial restriction map were derived. For the construction, *lacZ* (3.3 kb) cDNA lacking 3'UTR was ligated inframe to the initiation codon (ATG) at the 2nd exon of *Taz* locus by recombinant PCR technique, followed *loxP* flaked neomycin resistant gene with polyadenylation signal driven by the *Pgk1* promoter. The *lacZ-loxP-neo-loxP* was flanked by two homologous fragments of *Taz* genomic DNA length of 4.2 and 3.1 kb. Finally the construct was cloned in a plasmid containing a diphtheria toxin-A fragment (DT-A) gene driven by MC1 promoter for negative selection as described by Yagi *et al.*,(1993). A schematic representation of the *TAZ* loci and the homologous recombination construct is shown in Figure 1.

ES cell culture and transfection:

W4 ES cells (Taconic, USA) were cultured according to the standard protocol and electroporated with *SalI* linearized targeting vectors (Riele et al (1992) Proc Natl Acad Sci USA 89, 5128-5132); then cultured and selected against 200-300ug of G418. After the positive-negative selection, 120 ES colonies were picked-up and screened by long PCR technique using primers (p3 and p4, generating a 3.8 kb fragment from targeted allele). PCR positive clones were further examined by the southern hybridization for homologous recombination.

Chimera mice production and breeding:

ES cells from targeted clones were injected into blastocyst (3.5 dpc) embryos. One out of 4 clones had colonized the germ line of chimera mice. The chimera was then mated with wild type mice either with 129 or C57BL/6 strain and heterozygous offspring were identified by routine PCR technique using primers (p3 and p5, generating a 700 bp fragment from mutant allele). Heterozygous mice were further mated for the generation of

homozygous mutants and were genotyped using primers (p3 and p5) and primers (p1 and p3, generating a 500 bp fragment from wild type allele).

X-gal staining of kidney

The kidney was dissected from 13.5 to 15.5 days post coitum (dpc) and the lacZ staining was performed according to Lobe et al (1999) Dev Biol 208, 281-292. Stained embryos were washed three times with PBS and refixed in 4% paraformaldehyde in PBS. Samples were subsequently dehydrated and embedded in paraplast. Serial sections (4-8 mm) were prepared and counterstained with 1% eosin.

Histological analysis of organs

Organs from adult mice were fixed with 10% formalin at room temperature for 18-24 hours. Specimens were subsequently dehydrated and embedded in paraplast. Serial sections (4-8 mm) were prepared and stained with hematoxylin and eosin.

Survivality analysis:

Heterozygous mice were allowed to mate. The number of newborn was counted and subsequently dead pups were separated and genotyped routinely. However, surviving pups were genotyped at 3-4 weeks by PCR using tail DNA

Discussion

Transcriptional co-activator with PDZ-binding motif (TAZ) is a recently identified transcriptional co-activator with high homology to Yes associated protein (YAP) (Kanai et al, *supra*). TAZ is highly expressed in heart, lung, liver and kidney and functions as a transcriptional co-activator by binding, via a single WW domain, to PPXY motifs present in target transcription

factors (Kanai et al, *supra*). To date, bone-specific transcription factor (Cbfa1/Runx2) (Cui et al, *supra*) and thyroid transcription factor-1 (TTF-1/Nkx-2.1) (Park et al, *supra*) are the only known targets of TAZ. In addition to its association with transcription factors, TAZ can bind signaling and scaffolding proteins such as 14-3-3 and PDZ-domain proteins, interactions that are thought to regulate the subcellular localization of TAZ (Kanai et al, *supra*).

We inactivated by homologous recombination the TAZ gene in mice. Although $TAZ^{-/-}$ animals show an increased postnatal mortality, many live for at least 6 months, see Table 1 below:

Table 1: Survivality of *Taz* mutants

	Number of offspring	***Genotyping***			
		+/+	+/-	-/-	unknown
Live	92	24	54	14	0
Dead	15	2	0	8	5
Total	107	26	54	22	5
(%)	(100)	(24.3)	(50.5)	(20.5)	(4.7)

Surprisingly, the most striking phenotype of $TAZ^{-/-}$ mice are the slightly enlarged, anemic and cystic kidneys (Fig. 2). Renal cysts are restricted to the croticomedullary interface and absent from other organs, reminiscent of human NPH-MCKD (Figure 5). Given the autosomal recessive transmission and early onset (Hildebrandt et al *supra*), the phenotype of the $TAZ^{-/-}$ mice most closely relates to NPH.

The $TAZ^{-/-}$ animals express β-galactosidase under the control of the endogenous TAZ promoter. β-galactosidase staining in $TAZ^{-/-}$ and $TAZ^{+/-}$

mice and immunohistochemistry using TAZ antibodies in $TAZ^{+/-}$ and $TAZ^{+/+}$ animals indicate that TAZ is selectively expressed in the medullary tubules. TAZ expression in the kidney is first detected at embryonic stage 14.5dpc and the first indications of cyst development are apparent at 16.5dpc (Figure 6; $TAZ^{+/-}$ animals give the same staining of β-galactosidase activity as $TAZ^{-/-}$ but weaker. No staining is observed in the $TAZ^{+/+}$ animals control animals).

Example 2: Identifying further polypeptides regulated by TAZ

Identification of up- or downregulated genes in $TAZ^{-/-}$ mice

To identify differentially expressed genes which are up- or downregulated in TAZ mutant animals, microarrays are probed with RNA from kidneys of $TAZ^{-/-}$ and $TAZ^{+/-}$ littermates. RNA is isolated from whole kidney and medulla, or from individual cells that normally express TAZ.

Since TAZ expression is first detected at 14.5dpc and indications of cyst development are apparent at 16.5dpc (see above), analysis is performed with RNA isolated from whole kidney or dissected medulla (where TAZ is selectively expressed) from three stages: 14.5dpc (first detection of TAZ expression); 15.5dpc (TAZ expression and no cysts) and 16.5dpc (cyst development). In addition to kidney, arrays are probed with RNA from liver and pancreas of $TAZ^{-/-}$ and $TAZ^{+/-}$ mice. Since TAZ is highly expressed in liver and pancreas but these organs do not develop cysts in the $TAZ^{-/-}$ animals, comparison of data from kidney with that form liver and pancreas helps to narrow down differentially expressed genes relevant to kidney development and possibly cyst formation.

Since $TAZ^{-/-}$ and $TAZ^{+/-}$ mice express β-galactosidase under the control of the endogenous TAZ promoter (see above), detection of β-galactosidase activity, in conjunction with immunohistochemistry using markers specific for individual cell types, allows the identification of the cell type(s) in which TAZ is normally expressed. Microarray analysis can be performed using RNA probes from matched individual cells isolated by laser dissection microscopy (Hahn, S., Zhong, X.Y., and Holzgreve, W. (2002). Single cell PCR in laser capture microscopy. Methods Enzymol 356, 295-301 and Hahn, S., Zhong, X.Y., Troeger, C., Burgemeister, R., Gloning, K., and Holzgreve, W. (2000). Current applications of single-cell PCR. Cell Mol Life Sci 57, 96-105).

Comparative analysis of the microarray data sets for organs susceptible or not to cyst formation, for whole kidney, medulla and individual renal cells, and for different developmental stages, provides a detailed gene expression profiling for the critical phase between TAZ expression and cyst formation. In addition, this facilitates the identification of genes linked to cyst development. Genes of interest are identified based on specific criteria (see below) and their differential expression in kidneys of $TAZ^{-/-}$ and $TAZ^{+/-}$ mice confirmed by RT-PCR, *in situ* hybridization and, if antibodies are available, immunohistochemistry.

Identification of proteins that interact with TAZ

Yeast two-hybrid screens preformed using TAZ (both as full length protein or fragments encoding particular domains or domain combinations) as bait identifies interacting proteins encoded by a kidney cDNA library. The interaction between TAZ and identified prey proteins are confirmed both *in vitro* (pull down experiments using GST-TAZ fusion proteins and *in vitro* translated prey proteins) and *in vivo* (co-precipitation and co-localization

experiments following co-expression of epitope tagged TAZ and the prey). In addition to Cbfa (Cui et al, *supra*) and TTF-1 (Park et al, *supra*), both known to bind TAZ, additional transcription factors, as well as regulatory and structural proteins containing WW binding motifs or PDZ domains are identified.

Characterization of selected differentially expressed gene products and interacting proteins

Proteins encoded by genes differentially expressed in $TAZ^{-/-}$ versus $TAZ^{+/-}$ mice (see above) or proteins that interact with TAZ (see above) are further characterized to elucidate their function in normal kidney physiology and to determine how they might contribute to cyst formation.

Priority is given to proteins where the orthologue is mutated in patients suffering from NPH-MCKD. Linkage analysis in families with NPH-MCKD provides a multitude of positional candidate regions. This may rapidly identify novel human genes involved in NPH-MCKD.

Since positional candidate regions are only known for a relatively small number of NPH-MCKD patients, many differentially expressed genes or genes encoding interacting proteins are associated with such regions. Other criteria, such as a similar temporal and spatial expression in the kidney as TAZ is used to shortlist proteins to be characterized. Since cyst formation may be linked to abnormalities in epithelial cell growth, trans-epithelial transport, cell adhesion or primary cilia function, the focus is on proteins linked to these functions. Novel proteins of unknown function are considered. The details for the cell biological characterization of a selected protein will strongly depend on the function it is putatively associated with. Both *in vitro* cell culture models and *in vivo* animals models are used.

For *in vitro* experiments, use is made of kidney epithelial cell lines and the ability to grow these cells as monolayers (Hunziker et al (1991) Cell 66, 907-920) or organotypic cysts and tubules (Zegers et al (2003) Trend Cell Biol 13, 169-176). Expression of wild-type proteins or proteins in which particular domains have been mutated is studied in these culture systems with respect to subcellular localization and function. Furthermore, RNAi approaches are used to repress the expression of the protein. If antibodies to a particular protein are not available, epitope tagged variants are used.

For *in vivo* studies, use is made of of Zebrafish and mice as model systems. If there is a zebrafish ortholog for the protein of interest, *in situ* hybridization is used, or if working antibodies are available immunohistochemistry, to determine the temporal and spatial expression pattern in Zebrafish. The use morpholinos to repress expression of a particular gene in Zebrafish will allow to rapidly screen for kidney phenotypes in this animal model. The physiological role of chosen proteins is further defined in knock out mice, focusing on proteins whose gene is mutated in patients with HPH-MCKD.

CLAIMS

1. A non-human animal which has a reduced amount of functional TAZ polypeptide and/or TAZ-like polypeptide, or a reduced amount of nucleic acid encoding said polypeptide.

2. The non-human animal of claim 1 wherein the animal develops PKD.

3. The non-human animal of claim 1 or 2 wherein the animal is genetically modified.

4. The non-human animal of any one of the previous claims wherein the reduction in amount of functional TAZ polypeptide and/or TAZ-like polypeptide or nucleic acid encoding said polypeptide is localised to the kidney.

5. The non-human animal of any one of the previous claims wherein the animal is a rodent.

6. The non-human animal of any of the previous claims wherein the animal is a mouse.

7. The non-human animal according to any one of the previous claims wherein the animal has a reduced amount of functional native TAZ polypeptide or a reduced amount of nucleic acid encoding said polypeptide.

8. The non-human animal according to any one of claims 1 to 6 wherein the animal has a reduced amount of functional native YAP polypeptide or a reduced amount of nucleic acid encoding said polypeptide.

9. The non-human animal according to any one of claims 1 to 6 wherein the animal has a reduced amount of functional native TAZ and a reduced amount of functional native YAP polypeptide or a reduced amount of nucleic acid encoding said polypeptides.

10. The non-human animal according to any one of claims 1 to 7 and 9 wherein the animal has no functional TAZ polypeptide or no nucleic acid encoding said polypeptide.

11. A method for generating a non-human animal which develops PKD comprising reducing the amount of functional TAZ polypeptide and/or TAZ-like polypeptide, or reducing the amount of nucleic acid encoding said polypeptide.

12. The method of claim 11 wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by mutated one or more gene(s) encoding TAZ and/or TAZ-like polypeptide by homologous recombination.

13. The method of claim 11 wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by mutated one or more gene(s) encoding TAZ and/or TAZ-like polypeptide by insertional mutagenesis.

14. The method of claim 11 wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by mutated one or

more gene(s) encoding TAZ and/or TAZ-like polypeptide by chemical or physical mutagenesis.

15. The method of claim 11 wherein the amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide is reduced using antisense.

16. The method of claim 11 wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by supplying the animal with one or more agents that act as an antagonist of TAZ and/or a TAZ-like polypeptide.

17. The method of claim 11 wherein the amount of nucleic acid encoding TAZ polypeptide and/or a TAZ-like polypeptide is reduced by modifying the chromatin structure at or adjacent to a gene encoding a TAZ polypeptide and/or a TAZ-like polypeptide.

18. The method of claim 11 wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by modifying the function of an upstream regulator of TAZ polypeptide and/or a TAZ-like polypeptide.

19. The method of claim 11 wherein the amount of functional TAZ polypeptide and/or a TAZ-like polypeptide is reduced by supplying the animal with a dominant inactive form of a TAZ and/or a TAZ-like polypeptide.

20. A method for generating a non-human animal which develops PKD comprising:

i) identifying a polypeptide regulated by TAZ or a TAZ-like polypeptide, or a nucleic acid encoding said polypeptide;

ii) generating a non-human animal which has a reduced amount of the polypeptide and/or nucleic acid identified in step i); and optionally,

iii) selecting a non-human animal which develops PKD.

21. A non-human animal obtained or obtainable from the method of claim 20.

22. A method of screening for compounds of use in preventing or treating PKD wherein a non-human animal is administered a test compound and the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide, is assessed.

23. The method of claim 22 wherein the animal is a genetically modified non-human animal according to any one of claims 1 to 10 or 21.

24. A method of screening for compounds of use in preventing or treating PKD wherein a cell having TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ function is treated with a test compound and the effect of the test compound on the amount or function of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide, is assessed.

25. A method of identifying a compound of use in preventing or treating PKD comprising:

i) administering a test compound to a non-human animal;

ii) assessing the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of nucleic acid encoding said polypeptide; and,

iii) selecting any compound that increases the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or increases the amount of nucleic acid encoding said polypeptide.

26. A method of identifying a compound of use in preventing or treating PKD comprising:

i) administering a test compound to TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ;

ii) assessing the effect of the test compound on the function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide; and,

iii) selecting any compound that increases the function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide.

27. A method of identifying a compound of use in preventing or treating PKD comprising:

i) administering a test compound to cell comprising TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide;

ii) assessing the effect of the test compound on the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of a nucleic acid encoding said polypeptide; and,

iii) selecting any compound that increases the amount or function of TAZ polypeptide and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, or the amount of a nucleic acid encoding said polypeptide.

28. A method of identifying a compound of use in modulating PKD progression comprising:

i) administering a test compound to a non-human animal according to any one of the claims 1 to 10 and 21;

ii) assessing the effect of the test compound on PKD; and,

iii) selecting any compound that modulates PKD progression in the animal.

29. The method of claim 28 wherein the compound selected in step iii) reduces PKD progression.

30. The method of any one of claims 22 to 29 wherein a compound for use in preventing or treating PKD is formulated into a pharmaceutically acceptable composition.

31. A method of making a compound useful in preventing or treating PKD comprising synthesising a compound identified or selected by any one of claims 22 to 29.

32. A method of making a pharmaceutical composition comprising the method as claimed in any one of claims 22 to 29 and 31 and the step of mixing the compound of use in preventing or treating PKD with a pharmaceutically acceptable carrier.

33. A method for assessing a patient's risk of developing PKD or progression of PKD, comprising the steps of:

(i) obtaining a sample containing nucleic acid and/or protein from the patient; and,

(ii) determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the patient's genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide.

34. The method of claim 33 wherein if the sample has a reduced amount and/or function and/or an abnormal location of a TAZ polypeptide and/or

TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, or if the sample has a reduced amount of nucleic acid encoding TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, or if the sample has a deleterious mutation in one or more gene(s) encoding TAZ and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or TAZ-like polypeptide, then the patient is considered to be at risk of developing PKD.

35. A method according to claim 33 or 34 wherein the sample contains cells from the kidney.

36. A gene therapy vector comprising a polynucleotide which encodes TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide.

37. The gene therapy vector of claim 36 wherein the vector comprises a Moloney Leukaemia Virus (MLV) based retroviral vector or a lentiviral vector or adeno-associated vector (AAV).

38. A pharmaceutical composition comprising a polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or a compound identified from the methods of any one of claims 22 to 29 or a compound as defined in relation to claim 31 or a gene therapy vector as defined in claim 36 or 37 and a pharmaceutically acceptable carrier.

39. A polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like

polypeptide or a compound identified from the methods of any one of claims 22 to 29 or a compound as defined in relation to claim 31 or a pharmaceutical composition as defined in relation to any one of claims 30, 32 or 38 or a gene therapy vector as defined in claim 36 or 37 for use in medicine.

40. Use of a polynucleotide encoding TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide TAZ or a TAZ-like polypeptide or a polypeptide regulated by TAZ or a TAZ-like polypeptide or a compound identified from the methods of any one of claims 22 to 29 or a compound as defined in relation to claim 31 or a pharmaceutical composition as defined in relation to any one of claims 30, 32 or 38 or a gene therapy vector as defined in claim 36 or 37 in the manufacture of a medicament for the treatment of a patient with or at risk of developing PKD.

41. Use of an agent which is capable of determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide, and/or determining the genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide in the manufacture of a reagent for diagnosing PKD.

42. A method of assessing the status of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ in a patient comprising the steps of:

(i) obtaining a sample containing nucleic acid and/or protein from a patient; and,

(ii) determining the amount and/or function and/or location of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the amount of nucleic acid encoding TAZ and/or a TAZ-like polypeptide and/or a polypeptide regulated by TAZ or a TAZ-like polypeptide, and/or determining the patient's genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide.

43. A kit of parts useful for diagnosing PKD comprising an agent which is capable of use in assessing the amount or function of TAZ polypeptide and/or TAZ-like polypeptide and/or polypeptide regulated by TAZ, or the amount of nucleic acid encoding said polypeptide, or assessing the genotype of TAZ and/or a TAZ-like gene and/or a gene regulated by TAZ or a TAZ-like polypeptide in a sample.

44. A kit of parts according to Claim 43 further comprising a negative control and/or a positive control.

45. A kit of parts according to any one of Claims 43 or 44 further comprising means for separating kidney cells from a sample.