(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018702 A2

(51) International Patent Classification⁷: C12Q

(21) International Application Number: PCT/US2003/026507

(22) International Filing Date: 26 August 2003 (26.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/406,001 26 August 2002 (26.08.2002) US

(71) Applicant *(for all designated States except US)*: **THE REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; 300 South State Street, Ann Arbor, MI 48109-1280 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HILDEBRANDT, Friedhelm** [DE/US]; 580 Riverview, Ann Arbor, MI 48104 (US). **OTTO, Edgar** [DE/US]; 1111 Arella Boulevard, Ann Arbor, MI 48103 (US). **HOEFELE, Julia** [DE/US]; 514 E. Kingsley Street, Ann Arbor, MI 48104 (US). **RUF, Rainer** [DE/US]; 508 Lawrence Street, Ann Arbor, MI 48104 (US). **MUELLER, Adelheid, M.** [DE/US]; 8240 MSRB III, Ann Arbor, MI 48109 (US). **HILLER, Karl, S.** [DE/US]; 3660 Windemere Drive, Ann Arbor, MI 48105 (US). **WOLF, Matthias, T., F.** [DE/US]; 1111 Arella Boulevard, Ann Arbor, MI 481034 (US). **SCHUERMANN, Maria, J.** [DE/US]; 8240 MSRB III, Ann Arbor, MI 48109 (US). **BECKER, Achim** [DE/US]; 8420 MSRB III, Ann Arbor, MI 48109 (US).

(74) Agents: CASIMIR, David, A. et al.; Medlen & Carroll, LLP, 101 Howard Street, Suite 350, San Francisco, CA 94105 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NPHP NUCLEIC ACIDS AND PROTEINS

(57) Abstract: The present invention relates to Nephronophthisis, in particular to the NPHP4 protein (nephroretinin or nephrocystin-4) and nucleic acids encoding the NPHP4 protein. The present invention also provides assays for the detection of NPHP4, and assays for detecting nephroretinin and inversin polymorphisms and mutations associated with disease states.

NPHP NUCLEIC ACIDS AND PROTEINS

The present invention claims priority to U.S. Provisional Patent Application Serial Number 60/406,001, filed August 26, 2002, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0001] The present invention relates to Nephronophthisis, in particular to the NPHP4 protein (nephroretinin or nephrocystin-4) and nucleic acids encoding the NPHP4 protein. The present invention also provides assays for the detection of *NPHP4,* and assays for detecting nephroretinin and inversin polymorphisms and mutations associated with disease states.

BACKGROUND OF THE INVENTION

[0002] Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, constitutes the most frequent genetic cause for end-stage renal disease (ESRD) in children and young adults. NPHP is a progressive hereditary kidney disease marked by anemia, polyuria, renal loss of sodium, progressing to chronic renal failure, tubular atrophy, interstitial fibrosis, glomerular sclerosis, and medullary cysts.

[0003] The most prominent histologic feature of NPHP consists of renal fibrosis, which in chronic renal failure, regardless of origin, represents the pathogenic event correlated most strongly to loss of renal function (Zeisberg *et al.*, Hypertens. 10:315 [2001]). Therefore, NPHP has been considered a model disease for the development of renal fibrosis. The only treatment for NPHP is renal replacement therapy for survival (Smith *et al.*, Am. J. Dis. Child. 69:369 [1945]; Fanconi *et al.*, Helv. Paediatr. Acta. 6:1 [1951]; Hildebrandt, (1999) Juvenile nephronophthisis. In: Avner E, Holliday M, Barrat T (eds.) Pediatric Nephrology. Williams & Wilkins, Baltimore).

[0004] Three distinct gene loci for nephronophthisis, *NPHP1* [MIM 256100], *NPHP2* [MIM602088], and *NPHP3* [MIM 604387], have been mapped to chromosomes 2q13 (Antignac *et al.*, Nature Genet. 3:342 [1993]; Hildebrandt *et al.*, Am J Hum Genet 53:1256-1261 [1993]), 9q22 (Haider *et al.*, Am J Hum Genet 63:1404-1410 [1998), and 3q22 (Omran *et al.*, Am J Hum Genet 66:118–127 [2000]), respectively. These disease variants share renal histology of interstitial infiltrations, renal tubular cell atrophy with cyst development, and renal interstitial fibrosis (Waldherr *et al.*, Virchows Arch A Pathol Anat Histol 394:235–254 [1982]). The variants can be distinguished clinically by age of onset at ESRD. Renal failure develops at median ages of 1 year, 13 years, and 19 years, in NPHP2, NPHP1, and NPHP3, respectively (Omran *et al.*, [2000], *supra*).

[0005] Clearly there is a great need for identification of the molecular basis of NPHP, as well as for improved diagnostics and treatments for NPHP.

SUMMARY OF THE INVENTION

[0006] The present invention relates to Nephronophthisis, in particular to the NPHP4 protein (nephroretinin or nephrocystin-4) and nucleic acids encoding the NPHP4 protein. The present invention also provides assays for the detection of *NPHP4,* and assays for detecting nephroretinin and inversin polymorphisms and mutations associated with disease states.

[0007] Accordingly, in some embodiments, the present invention provides an isolated and purified nucleic acid comprising a sequence encoding a protein selected from the group consisting of SEQ ID NOs: 2, 6, 8, 10, 12, 14, 16, 18, and 20. In some embodiments, the sequence is operably linked to a heterologous promoter. In some embodiments, the sequence is contained within a vector. In some embodiments, the vector is within a host cell. In some embodiments, the present invention provides a computer readable medium encoding a representation of the nucleic acid sequence.

[0008] The present invention also provides an isolated and purified nucleic acid sequence that hybridizes under conditions of low stringency to a nucleic acid selected from the group consisting of SEQ ID NOs: 1, 5, 7, 9, 11, 13, 15, 17, and 19. In some embodiments, the sequence is contained within a vector. In some embodiments, the vector is in a host cell. In some embodiments, the host cell is located in an organism, wherein the organism is a non-human animal.

[0009] The present invention additionally provides a protein encoded by a nucleic acid selected from the group consisting of SEQ ID NOs:1 and variants thereof that are at least 80% identical to SEQ ID NOs: 1 5, 7, 9, 11, 13, 15, 17, and 19. In some embodiments, the protein is at least 90%, and preferably at least 95% identical to SEQ ID NOs: 1, 5, 7, 9, 11, 13, 15, 17, and 19. In some embodiments, the present invention provides a computer readable medium encoding a representation of the polypeptide sequence.

[0010] The present invention further provides a composition comprising a nucleic acid that inhibits the binding of at least a portion of a nucleic acid selected from the group consisting of SEQ ID NOs:1, 5, 7, 9, 11, 13, 15, 17, and 19 to their complementary sequences. In other embodiments, the present invention provides a polynucleotide sequence comprising at least fifteen nucleotides capable of hybridizing under stringent conditions to the isolated nucleotide sequence.

[0011] In yet other embodiments, the present invention provides a composition comprising a variant nephroretinin polypeptide, wherein the polypeptide comprises a C-terminal truncation of SEQ ID NO:2. In some embodiments, the variant nephroretinin polypeptide is selected from the group consisting of SEQ ID NOs: 6, 10, 12, 14, 16, and 20. In some embodiments, the presence of the variant polypeptide in a subject is indicative of nephronophthisis type 4 kidney disease in the subject.

[0012] In still further embodiments, the present invention provides a method for detection of a variant nephroretinin polypeptide in a subject, comprising: providing a biological sample from a subject, wherein the biological sample comprises a nephroretinin polypeptide; and detecting the presence or absence of a variant nephroretinin polypeptide in the biological sample. In some embodiments, the variant nephroretinin polypeptide is a C-terminal truncation of SEQ ID NO:2.

In some embodiments, the variant nephroretinin polypeptide is selected from the group consisting of SEQ ID NOs: 6, 10, 12, 14, 16, and 20. In some embodiments, the presence of the variant nephroretinin polypeptide is indicative of nephronophthisis type 4 kidney disease in the subject. In some embodiments, the biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample. In some embodiments, the subject is selected from the group consisting of an embryo, a fetus, a newborn animal, and a young animal. In some embodiments, the animal is a human. In some embodiments, the detecting comprises differential antibody binding. In other embodiments, the detecting comprises a gel-free truncation test. In still other embodiments, the detection comprises a Western blot.

[0013] The present invention further provides a kit comprising a reagent for detecting the presence or absence of a variant nephroretinin polypeptide in a biological sample. In some embodiments, the kit further comprises instruction for using the kit for detecting the presence or absence of a variant nephroretinin polypeptide in a biological sample. In some embodiments, the instructions comprise instructions required by the U.S. Food and Drug Agency for *in vitro* diagnostic kits. In some embodiments, the kit further comprises instructions for diagnosing nephronophthisis in the subject based on the presence or absence of the variant nephroretinin polypeptide. In some embodiments, the nephronophthisis is nephronophthisis type 4. In some embodiments, the reagent is one or more antibodies. In some embodiments, the antibodies comprise a first antibody that specifically binds to the C-terminus of the nephroretinin polypeptide and a second antibody that specifically binds to the N-terminus of the nephroretinin polypeptide. In other embodiments, the reagents comprise reagents for performing a gel-free truncation test. In some embodiments, the variant nephroretinin polypeptide is a C-terminal truncation of SEQ ID NO:2, for example, in some embodiments, the variant nephroretinin polypeptide is selected from the group consisting of SEQ ID NOs: 6, 10, 12, 14, 16, and 20. In some embodiments, the biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample.

[0014] In still further embodiments, the present invention provides a method for detection of a variant inversin polypeptide in a subject, comprising: providing a biological sample from a subject, wherein the biological sample comprises a inversin polypeptide; and detecting the presence or absence of a variant inversin polypeptide in the biological sample. In some embodiments, the variant inversin polypeptide is a C-terminal truncation of SEQ ID NO:22. In some embodiments, the variant inversin polypeptide is selected from the group consisting of SEQ ID NOs: 24, 26, 28, 30, 34, 36, 38 and 40. In some embodiments, the presence of the variant inversin polypeptide is indicative of nephronophthisis type 2 kidney disease in the subject. In some embodiments, the biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample. In some embodiments, the subject is selected from the group consisting of an embryo, a fetus, a newborn animal, and a young animal. In some embodiments, the animal is a human. In some embodiments, the detecting comprises differential antibody binding. In other embodiments, the detecting comprises a gel-free truncation test. In still other embodiments, the detection comprises a Western blot.

[0015] The present invention also provides a kit comprising a reagent for detecting the presence or absence of a variant inversin polypeptide or nucleic acid in a biological sample. In further embodiments, the kit further comprises reagents for detecting the presence or absence of a variant nephroretinin polypeptide or nucleic acid, or a variant nephrocystin-3 polypeptide or nucleic acid. In some embodiments, the kit further comprises instruction for using the kit for detecting the presence or absence of a variant inversin polypeptide or nucleic acid in a biological sample. In some embodiments, the instructions comprise instructions required by the U.S. Food and Drug Agency for *in vitro* diagnostic kits. In some embodiments, the kit further comprises instructions for diagnosing nephronophthisis in the subject based on the presence or absence of the variant inversin polypeptide or nucleic acid. In some embodiments, the kit further comprises instructions for diagnosing nephronophthisis in the subject based on the presence or absence of the variant inversin polypeptide or nucleic acid, the variant nephroretinin polypeptide or nucleic acid, or the variant nephrocystin-3 polypeptide or nucleic acid. In some embodiments, the nephronophthisis is nephronophthisis type 2. In other embodiments, the nephronophthisis is nephronophthisis type 2, nephronophthisis type 4, or nephronophthisis type 3. In some embodiments, the reagent is one or more antibodies. In some embodiments, the antibodies comprise a first antibody that specifically binds to the C-terminus of the inversin polypeptide and a second antibody that specifically binds to the N-terminus of the inversin polypeptide. In other embodiments, the reagents comprise reagents for performing a gel-free truncation test. In some embodiments, the variant inversin polypeptide is a C-terminal truncation of SEQ ID NO:22, for example, in some embodiments, the variant inversin polypeptide is selected from the group consisting of SEQ ID NOs: 24, 26, 28, 30, 34, 36, 38 and 40. In some embodiments, the biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample.

DESCRIPTION OF THE FIGURES

[0016] Figure 1 shows haplotype results on chromosome 1p36 carried out for refining the *NPHP4* locus in affected offspring from 3 consanguineous NPHP families. p-ter, telomeric; cen, centromeric; nd, not done.

[0017] Figure 2 shows the positional cloning strategy for the *NPHP4* gene on human chromosome 1p36. Figure 2*A*, genetic map position for microsatellites used in linkage mapping of *NPHP4* (see Fig. 1). Published flanking markers are underlined (Schuermann *et al.*, Am. J. Hum. Genet. 70:1240 [2002]. p-ter, telomeric; cen, centromeric. Figure 2*B*, physical map distances of critical microsatellites relative to *D1S2660*. The secure 1.2 Mb critical interval (solid bar) and the 700 kb suggestive critical interval (stippled bar), are shown delimited by the newly identified secure flanking markers (asterisks) and suggestive flanking markers (double asterisks) defined by haplotype analysis (see fig. 1). Below the axis known genes, predicted unkown genes, and the *NPHP4* gene (alias *Q9UFQ2*) are represented as arrows in the direction of transcription. Figure 2*C*, genomic organization of *NPHP4* with exons indicated as vertical hatches and numbered. Figure 2*D*, exon structure of *NPHP4* cDNA. Black and white boxes represent the 30 exons encoding nephroretinin. The number of the first codon of each exon is indicated; exons beginning with the second or third base of a codon are indicated by "b" or "c", respectively. At the bottom locations of the 11 different mutations identified in 8 *NPHP* kindred are shown. fs, frameshift. Figure 2*E*, *NPHP4* mutations occurring homozygously in affecteds of 5 consanguineous families (underlined). Mutated nucleotides and altered amino acids are depicted on grey background.

[0018] Figure 3 shows Northern blot analysis of the *NPHP4* expression pattern. Expression of a 5.9 kb transcript (arrowhead) is apparent in all tissues studied with highest expression in skeletal muscle.

[0019] Figure 4 shows the nucleic acid (cDNA) (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences of NPHP4.

[0020] Figure 5 shows an alignment of human (SEQ ID NO: 2), mouse (SEQ ID NO: 3), and *C. elegans* (SEQ ID NO: 4) NPHP4 amino acid sequences.

[0021] Figure 6 shows the nucleic acid (SEQ ID NO: 5) and amino acid (SEQ ID NO: 6) sequences of an exemplary NPHP4 variant found in family 3 (See Table 1).

[0022] Figure 7 shows the nucleic acid (SEQ ID NO: 7) and amino acid (SEQ ID NO:8) sequences of an exemplary NPHP4 variant found in family 24 (See Table 1).

[0023] Figure 8 shows the nucleic acid (SEQ ID NO: 9) and amino acid (SEQ ID NO:10) sequences of an exemplary NPHP4 variant found in family 30 (See Table 1).

[0024] Figure 9 shows the nucleic acid (SEQ ID NO: 11) and amino acid (SEQ ID NO:12) sequences of an exemplary NPHP4 variant found in family 32 (See Table 1).

[0025] Figure 10 shows the nucleic acid (SEQ ID NO: 13) and amino acid (SEQ ID NO:14) sequences of an exemplary NPHP4 variant found in family 60 (See Table 1).

[0026] Figure 11 shows the nucleic acid (SEQ ID NO: 15) and amino acid (SEQ ID NO: 16) sequences of an exemplary NPHP4 variant found in family 461 (See Table 1).

[0027] Figure 12 shows the nucleic acid (SEQ ID NO: 17) and amino acid (SEQ ID NO: 18) sequences of an additional exemplary NPHP4 variant found in family 461 (See Table 1).

[0028] Figure 13 shows the nucleic acid (SEQ ID NO: 19) and amino acid (SEQ ID NO:20) sequences of an exemplary NPHP4 variant found in family 622 (See Table 1).

[0029] Figure 14 shows the nucleic acid (cDNA) (SEQ ID NO: 21) and amino acid (SEQ ID NO: 22) sequences of inversin.

[0030] Figure 15 shows mutations in *INVS* in individuals with NPHP2. Figures 2a and 2d show mutations in *INVS* (nucleotide exchange and amino acid exchange) together with sequence traces for mutated sequences (top) and sequence from healthy controls (bottom). Family numbers are given above boxes. Figure 2b shows the exon structure of *INVS*. Figure 2c shows a representation of protein motifs found in inversin. aa, amino acid residues; Ank, ankyrin/swi6 motif; D1, D box1 (Apc2-binding[23]); D2, D box2; IQ, calmodulin binding domains.

[0031] Figure 16 depicts the specific nucleotide exchange (SEQ ID NO: 23) and resulting termination of the amino acid sequence (SEQ ID NO: 24) of an exemplary inversin variant found in family A6 (See Table 3).

[0032] Figure 17 depicts a specific nucleotide deletion (SEQ ID NO: 25) and resulting termination of the amino acid sequence (SEQ ID NO: 26) of an exemplary inversin variant found in family A6 (See Table 3).

[0033] Figure 18 depicts the specific nucleotide exchange (SEQ ID NO: 27) and resulting termination of the amino acid sequence (SEQ ID NO: 28) of an exemplary inversin variant found in family A8 (See Table 3).

[0034] Figure 19 depicts the specific nucleotide exchange (SEQ ID NO: 29) and resulting termination of the amino acid sequence (SEQ ID NO: 30) of an exemplary inversin variant found in family A9 (See Table 3).

[0035] Figure 20 depicts the specific nucleotide exchange (SEQ ID NO: 31) and resulting substitution in the amino acid sequence (SEQ ID NO: 32) of an exemplary inversin variant found in family A9 (See Table 3).

[0036] Figure 21 depicts a specific nucleotide deletion (SEQ ID NO: 33) and resulting termination of the amino acid sequence (SEQ ID NO: 34) of an exemplary inversin variant found in family A10 (See Table 3).

[0037] Figure 22 depicts the specific nucleotide exchange (SEQ ID NO: 35) and resulting termination of the amino acid sequence (SEQ ID NO: 36) of an exemplary inversin variant found in family A12 (See Table 3).

[0038] Figure 23 depicts the specific nucleotide exchange (SEQ ID NO: 37) and resulting termination of the amino acid sequence (SEQ ID NO: 38) of an exemplary inversin variant found in family 868 (See Table 3).

[0039] Figure 24 depicts a specific nucleotide insertion (SEQ ID NO: 39) and resulting termination of the amino acid sequence (SEQ ID NO: 40) of an exemplary inversin variant found in family 868 (See Table 3).

[0040] Figure 25 depicts the specific nucleotide exchange (SEQ ID NO: 41) and resulting substitution in the amino acid sequence (SEQ ID NO: 42) of an exemplary inversin variant found in family A7 (See Table 3).

[0041] Figure 26 shows the association of inversin with nephrocystin in HEK 293T cells and in mouse tissue.

[0042] Figure 27 shows the molecular interaction of nephrocystin with β-tubulin.

[0043] Figure 28 shows the co-localization of nephrocystin and inversin to primary cilia in renal tubular epithelial cells.

[0044] Figure 29 shows the disruption of zebrafish *invs* function results in renal cyst formation.

GENERAL DESCRIPTION OF THE INVENTION

[0045] The gene for nephronophthisis type 1 (*NPHP1*) has been cloned by positional cloning (Hildebrandt *et al.*, Nature Genet 17:149–153 [1997]). Its gene product, nephrocystin, represents a novel docking protein, which interacts with the signaling proteins p130Cas, tensin, focal adhesion kinase 2, and filamin A and B, which are involved in cell-cell and cell-matrix signaling of renal epithelial cells (Hildebrandt and Otto, J Am Soc Nephrol 11:1753–1761 [2000]; Donaldson *et al.*, Exp Cell Res 256:168-178 [2000]; Benzing *et al.*, Proc Natl Acad Sci USA 98:9784–9789 [2001]; Donaldson *et al.*, J Biol Chem 277:29028-29035 [2002]). The association of NPHP with autosomal recessive retinitis pigmentosa (RP), has been described as the so-called Senior-Løken syndrome (SLS [MIM 266900]) (Senior *et al.*, Am J Ophthalmol 52:625–633 [1961]; Løken *et al.*, Acta Paediatr 50:177–184 [1961]; each of which is herein incorporated by reference). In families with SLS, linkage has been demonstrated to the loci for *NPHP1* and *NPHP3* (Caridi *et al.*, Am J Kidney Dis 32:1059–1062 [1998]; Omran *et al.*, 2002, *supra*). Very recently, a new gene locus (*NPHP4*) for NPHP type 4 (Schuermann *et al.*, Am. J. Hum. Genet. 70:1240 [2002]; herein incorporated by reference) has been identified and linkage of a large SLS kindred to this locus demonstrated.

[0046] Experiments conducted during the course of development of the present invention identified, by positional cloning, the gene (*NPHP4*) causing NPHP type 4, through demonstration of 9 likely loss-of-function mutations in 6 affected families. In addition, 2 loss of function mutations in patients from 2 families with SLS were detected. The conclusion that the gene cloned in the experiments described herein is the gene causing NPHP type 4 is based on identification, in 8 families with NPHP, of 9 distinct truncating mutations and 2 missense mutations, none of which occurred in over 92 healthy control individuals. Experiments conducted during the course of development of the present invention further demonstrated the presence of 2 homozygous truncating mutations also in 2 families with SLS (F3 and F60). A small percentage of patients also exhibit SLS in families with *NPHP1* mutations (Caridi *et al.*, Am. J. Kidney Disease 32:1059 [1998]) and in families linked to *NPHP3* (Omran *et al.* 2002, surpa). For all 3 genes no distinction can be made on the basis of allelic differences between the NPHP phenotypes with and without RP. Therefore, it seems likely that a stochastic pleiotropic effect is responsible for the occurrence of RP in NPHP types 1, 3 and 4. Accordingly, in some embodiments, the present invention provides the NPHP4 nucleic acid and amino acid sequence, as well as disease related variants therof.

[0047] *NPHP4* is a novel gene, which is unrelated to any known gene families. It encodes a novel protein, "nephroretinin" or "nephrocystin-4". *NPHP4*, like *NPHP1*, is unique to the human genome, is conserved in *C. elegans*, and exhibits a broad expression pattern. Identification of the *NPHP1* gene (Hildebrandt *et al.*, Nature Genet. 17:149 [1997]) revealed nephrocystin as a novel docking protein, which interacts with p130Cas (Donaldson *et al.*, Exp. Cell. Res. 256:168 [2000]; Hildebrandt and Otto, J. Am. Soc. Nephrol. 11:1753 [2000]), tensin, focal adhesion kinase 2 (Benzing *et al.*, PNAS 98:9784 [2001]), and filamin A and B (Donaldson *et al.*, 2002, *supra*), and which is involved in cell-cell and cell-matrix signaling. The present invention is not limited to a particular mechanism of action. Indeed, an understanding of the mechanism is not necessary to practice the present invention. Nonetheless, it is therefore likely that both nephroretinin and nephrocystin, interact within a novel shared pathogenic pathway. Thus, the present invention provides a novel gene with critical roles in renal tissue architecture and ophthalmic function.

[0048] Two additional gene loci have been mapped for NPHP. The locus *NPHP3* associated with adolescent NPHP localizes to human chromosome 3q22 (Omran, *et al.*, Am. J. Hum. Genet. 66, 118 [2000]), and *NPHP2* associated with infantile NPHP resides on chromosome 9q21–q22 (Haider *et al.*, Am. J. Hum. Genet. 63, 1404 [1998]). The kidney phenotype of NPHP2 combines features of NPHP, including tubular basement membrane disruption and renal interstitial fibrosis, with features of PKD (Gagnadoux *et al.*, Pediatr. Nephrol. 3, 50 [1989]) including enlarged kidneys and widespread cyst development. During the course of development of the present invention, the human gene *INVS* was determined to be located in the *NPHP2* critical genetic interval (Haider *et al.*, Am. J. Hum. Genet. 63, 1404 [1998]).

[0049] In the *inv/inv* mouse model of insertional mutagenesis, a deletion of exons 3–11 of *Invs* encoding inversin causes a phenotype of cyst formation in enlarged kidneys, *situs inversus* and pancreatic islet cell dysplasia (Mochizuki *et al.*, Nature 395, 177 [1998]; Morgan *et al.*, Nat. Genet. 20, 149 [1998]). Histology of infantile NPHP2 and of the *inv/inv* mouse identified features resembling NPHP, namely interstitial fibrosis, mild interstitial cell infiltration, tubular cell atrophy, tubular cysts and periglomerular fibrosis. In addition, human NPHP2 and mouse *inv/inv* phenotypes showed features reminiscent of autosomal dominant PKD, such as kidney enlargement, absence of the tubular basement membrane irregularity characteristic of NPHP and presence of cysts also outside the medullary region.

[0050] Experiments conducted during the course of development of the present invention identified the gene (*INVS*) causing NPHP type 2, through demonstration of 8 likely loss-of-function mutations in 6 affected families. The conclusion that the gene identified in the experiments described herein is the gene causing NPHP type 2 is based on identification, in 7 families with NPHP, of 8 distinct truncating mutations and 2 missense mutations, none of which occurred in over 100 healthy control individuals.

DEFINITIONS

[0051] To facilitate understanding of the invention, a number of terms are defined below.

[0052] As used herein, the term "NPHP4" or " nephroretinin" or "nephrocystin-4" when used in reference to a protein or nucleic acid refers to a protein or nucleic acid encoding a protein that, in some mutant forms, is correlated with nephronophthisis. The term NPHP4 encompasses both proteins that are identical to wild-type NPHP4 and those that are derived from wild type NPHP4 (*e.g.*, variants of NPHP4 or chimeric genes constructed with portions of NPHP4 coding regions). In some embodiments, the "NPHP4" is the wild type nucleic acid (SEQ ID NO: 1) or amino acid (SEQ ID NO:2) sequence. In other embodiments, the "NPHP4" is a variant or mutant (*e.g.*, including, but not limited to, the nucleic acid sequences described by SEQ ID NOS: 5, 7, 9, 11, 13, 15, 17, 19 and the amino acid sequences described by SEQ ID NOS: 6, 8, 10, 12, 14, 16, 18, and 20).

[0053] As used herein, the term "INVS" or "inversin" when used in reference to a protein or nucleic acid refers to a protein or nucleic acid encoding a protein that, in some mutant forms, is correlated with nephronophthisis. In some embodiments, the "inversin" is the wild type nucleic acid (SEQ ID NO: 21) or amino acid (SEQ ID NO:22) sequence. In other embodiments, the "inversin" is a variant or mutant (*e.g.*, including, but not limited to, the nucleic acid sequences described by SEQ ID NOS: 23, 25, 27, 29, 31, 33, 35, 37, and 39 and the amino acid sequences described by SEQ ID NOS: 24, 26, 28, 30, 32, 34, 36, 38 and 40).

[0054] As used herein, the term "C-terminal truncation of SEQ ID NO:2 refers to a polypeptide comprising a portion of SEQ ID NO:2, wherein the portion comprises the N-terminus of SEQ ID NO:2. In preferred embodiments, the N-terminal portion comprises at lease 200 amino acids, preferably at least 400 amino acids, and even more preferably at least 700 amino acids of SEQ ID NO:2. Exemplary C-terminal truncations of SEQ ID NO:2 include, but are not limited to, SEQ ID NOs: 6, 10, 12, 14, 16, and 20, and the term "C-terminal truncation of SEQ ID NO:22 refers to a polypeptide comprising a portion of SEQ ID NO:22, wherein the portion comprises the N-terminus of SEQ ID NO:22. In preferred embodiments, the N-terminal portion comprises at lease 200 amino acids, preferably at least 400 amino acids, and even more preferably at least 700 amino acids of SEQ ID NO:22. Exemplary C-terminal truncations of SEQ ID NO:22 include, but are not limited to, SEQ ID NOs: 24, 26, 28, 30, 34, 36, 38 and 40.

[0055] As used herein, the terms "instructions for using said kit for said detecting the presence or absence of a variant nephroretinin polypeptide in a said biological sample"or "instructions for using said kit for said detecting the presence or absence of a variant inversin polypeptide in a said biological sample" includes instructions for using the reagents contained in the kit for the detection of variant and wild type nephroretinin and inversin polypeptides, respectfully. In some embodiments, the instructions further comprise the statement of intended use required by the U.S. Food and Drug Administration (FDA) in labeling *in vitro* diagnostic products. The FDA classifies *in vitro* diagnostics as medical devices and requires that they be approved through the 510(k) procedure. Information required in an application under 510(k) includes: 1) The in vitro diagnostic product name, including the trade or proprietary name, the common or usual name, and the classification name of the device; 2) The intended use of the product; 3) The establishment registration number, if applicable, of the owner or operator submitting the 510(k) submission; the class in which the in vitro diagnostic product was placed under section 513 of the FD&C Act, if known, its appropriate panel, or, if the owner or operator determines that the device has not been classified under such section, a statement of that determination and the basis for the determination that the in vitro diagnostic product is not so classified; 4)Proposed labels, labeling and advertisements sufficient to describe the in vitro diagnostic product, its intended use, and directions for use. Where applicable, photographs or engineering drawings should be supplied; 5) A statement indicating that the device is similar to and/or different from other in vitro diagnostic products of comparable type in commercial distribution in the U.S., accompanied by data to support the statement; 6) A 510(k) summary of the safety and effectiveness data upon which the substantial equivalence determination is based; or a statement that the 510(k) safety and effectiveness information supporting the FDA finding of substantial equivalence will be made available to any person within 30 days of a written request; 7) A statement that the submitter believes, to the best of their knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted; 8) Any additional information regarding the in vitro diagnostic product requested that is necessary for the FDA to make a substantial equivalency determination. Additional information is available at the Internet web page of the U.S. FDA.

[0056] The term "gene" refers to a nucleic acid (*e.g.*, DNA) sequence that comprises coding sequences necessary for the production of a polypeptide, RNA (*e.g.*, including but not limited to, mRNA, tRNA and rRNA) or precursor (*e.g.*, NPHP4). The polypeptide, RNA, or precursor can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (*e.g.*, enzymatic activity, ligand binding, signal transduction, etc.) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and the including sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb on either end such that the gene corresponds to the length of the full-length mRNA. The sequences that are located 5' of the coding region and which are present on the mRNA are referred to as 5' untranslated sequences. The sequences that are located 3' or downstream of the coding region and that are present on the mRNA are referred to as 3' untranslated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA (mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

[0057] In particular, the term "NPHP4 gene" refers to the full-length NPHP4 nucleotide sequence (*e.g.*, contained in SEQ ID NO: 1). However, it is also intended that the term encompass fragments of the NPHP4 sequence, mutants (*e.g.*, SEQ ID NOS: 5, 7, 9, 11, 13, 15, 17, 21, 23, and 25) as well as other domains within the full-length NPHP4 nucleotide sequence. Furthermore, the terms "NPHP4 nucleotide sequence" or "NPHP4 polynucleotide sequence" encompasses DNA, cDNA, and RNA (*e.g.*, mRNA) sequences.

[0058] Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

[0059] In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences that are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region may contain regulatory sequences such as promoters and enhancers that control or influence the transcription of the gene. The 3' flanking region may contain sequences that direct the termination of transcription, post-transcriptional cleavage and polyadenylation.

[0060] The term "wild-type" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene. In contrast, the terms "modified," "mutant," "polymorphism," and "variant" refer to a gene or gene product that displays modifications in sequence and/or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[0061] As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

[0062] DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotides or polynucleotide, referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

[0063] As used herein, the terms "an oligonucleotide having a nucleotide sequence encoding a gene" and "polynucleotide having a nucleotide sequence encoding a gene," means a nucleic acid sequence comprising the coding region of a gene or, in other words, the nucleic acid sequence that encodes a gene product. The coding region may be present in a cDNA, genomic DNA, or RNA form. When present in a DNA form, the oligonucleotide or polynucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers/promoters, splice junctions, polyadenylation signals, *etc.* may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers/promoters, splice junctions, intervening sequences, polyadenylation signals, *etc.* or a combination of both endogenous and exogenous control elements.

[0064] As used herein, the term "regulatory element" refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements include splicing signals, polyadenylation signals, termination signals, etc.

[0065] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence 5'-"A-G-T-3'," is complementary to the sequence 3'-"T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

[0066] The term "homology" refers to a degree of complementarity. There may be partial homology or complete homology (*i.e.*, identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid and is referred to using the functional term "substantially homologous." The term "inhibition of binding," when used in reference to nucleic acid binding, refers to inhibition of binding caused by competition of homologous sequences for binding to a target sequence. The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous to a target under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target that lacks even a partial degree of complementarity (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

[0067] The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, etc.) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, etc.). Furthermore, when used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe that can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above.

[0068] A gene may produce multiple RNA species that are generated by differential splicing of the primary RNA transcript. cDNAs that are splice variants of the same gene will contain regions of sequence identity or complete homology (representing the presence of the same exon or portion of the same exon on both cDNAs) and regions of complete non-identity (for example, representing the presence of exon "A" on cDNA 1 wherein cDNA 2 contains exon "B" instead). Because the two cDNAs contain regions of sequence identity they will both hybridize to a probe derived from the entire gene or portions of the gene containing sequences found on both cDNAs; the two splice variants are therefore substantially homologous to such a probe and to each other.

[0069] When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

[0070] As used herein, the term "competes for binding" is used in reference to a first polypeptide with an activity which binds to the same substrate as does a second polypeptide with an activity, where the second polypeptide is a variant of the first polypeptide or a related or dissimilar polypeptide. The efficiency (*e.g.*, kinetics or thermodynamics) of binding by the first polypeptide may be the same as or greater than or less than the efficiency substrate binding by the second polypeptide. For example, the equilibrium binding constant (K_D) for binding to the substrate may be different for the two polypeptides. The term "K_m" as used herein refers to the Michaelis-Menton constant for an enzyme and is defined as the concentration of the specific substrate at which a given enzyme yields one-half its maximum velocity in an enzyme catalyzed reaction.

[0071] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids.

[0072] As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (*See e.g.*, Anderson and Young, Quantitative Filter

Hybridization, *in Nucleic Acid Hybridization* [1985]). Other references include more sophisticated computations that take structural as well as sequence characteristics into account for the calculation of T_m.

[0073] As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. Those skilled in the art will recognize that "stringency" conditions may be altered by varying the parameters just described either individually or in concert. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high frequency of complementary base sequences (*e.g.*, hybridization under "high stringency" conditions may occur between homologs with about 85-100% identity, preferably about 70-100% identity). With medium stringency conditions, nucleic acid base pairing will occur between nucleic acids with an intermediate frequency of complementary base sequences (*e.g.*, hybridization under "medium stringency" conditions may occur between homologs with about 50-70% identity). Thus, conditions of "weak" or "low" stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

[0074] "High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42 C when a probe of about 500 nucleotides in length is employed.

[0075] "Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42 C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42 C when a probe of about 500 nucleotides in length is employed.

[0076] "Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42 C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharamcia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42 C when a probe of about 500 nucleotides in length is employed. . The present invention is not limited to the hybridization of probes of about 500 nucleotides in length. The present invention contemplates the use of probes between approximately 10 nucleotides up to several thousand (*e.g.*, at least 5000) nucleotides in length.

[0077] One skilled in the relevant understands that stringency conditions may be altered for probes of other sizes (See *e.g.*, Anderson and Young, Quantitative Filter Hybridization, *in Nucleic Acid Hybridization* [1985] and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, NY [1989]).

[0078] The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA sequence given in a sequence listing or may comprise a complete gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (*i.e.*, a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a reference sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman [Smith and Waterman, *Adv. Appl. Math.* 2: 482 (1981)] by the homology alignment algorithm of Needleman and Wunsch [Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970)], by the search for similarity method of Pearson and Lipman [Pearson and Lipman, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988)], by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected. The term "sequence identity" means that two polynucleotide sequences are identical (*i.e.*, on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 25-50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison. The reference sequence may be a subset of a larger sequence, for example, as a segment of the full-length sequences of the compositions claimed in the present invention (*e.g.*, NPHP4).

[0079] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity or more (*e.g.*, 99 percent sequence identity). Preferably, residue positions that are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

[0080] The term "fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion as compared to the native protein, but where the remaining amino acid sequence is identical to the corresponding positions in the amino acid sequence deduced from a full-length cDNA sequence. Fragments typically are at least 4 amino acids long, preferably at least 20 amino acids long, usually at least 50 amino acids long or longer, and span the portion of the polypeptide required for intermolecular binding of the compositions (claimed in the present invention) with its various ligands and/or substrates.

[0081] The term "polymorphic locus" is a locus present in a population that shows variation between members of the population (*i.e.*, the most common allele has a frequency of less than 0.95). In contrast, a "monomorphic locus" is a genetic locus at little or no variations seen between members of the population (generally taken to be a locus at which the most common allele exceeds a frequency of 0.95 in the gene pool of the population).

[0082] As used herein, the term "genetic variation information" or "genetic variant information" refers to the presence or absence of one or more variant nucleic acid sequences (*e.g.*, polymorphism or mutations) in a given allele of a particular gene (*e.g.*, the NPHP4 gene).

[0083] As used herein, the term "detection assay" refers to an assay for detecting the presence of absence of variant nucleic acid sequences (*e.g.*, polymorphism or mutations) in a given allele of a particular gene (*e.g.*, the NPHP4 gene). Examples of suitable detection assays include, but are not limited to, those described below in Section III B.

[0084] The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

[0085] "Amplification" is a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (*i.e.*, replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (*i.e.*, synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out.

[0086] Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Qβ replicase, MDV-1 RNA is the specific template for the replicase (D.L. Kacian *et al.*, Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlin *et al.*, Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (D.Y. Wu and R. B. Wallace, Genomics 4:560 [1989]). Finally, *Taq* and *Pfu* polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), *PCR Technology*, Stockton Press [1989]).

[0087] As used herein, the term "amplifiable nucleic acid" is used in reference to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" will usually comprise "sample template."

[0088] As used herein, the term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is most often inadvertent. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

[0089] As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (*i.e.*, in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

[0090] As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, that is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

[0091] As used herein, the term "target," refers to a nucleic acid sequence or structure to be detected or characterized. Thus, the "target" is sought to be sorted out from other nucleic acid sequences. A "segment" is defined as a region of nucleic acid within the target sequence.

[0092] As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

[0093] With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

[0094] As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

[0095] As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

[0096] As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

[0097] As used herein, the term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

[0098] As used herein, the term "antisense" is used in reference to RNA sequences that are complementary to a specific RNA sequence (*e.g.*, mRNA). Included within this definition are antisense RNA ("asRNA") molecules involved in gene regulation by bacteria. Antisense RNA may be produced by any method, including synthesis by splicing the gene(s) of interest in a reverse orientation to a viral promoter that permits the synthesis of a coding strand. Once introduced into an embryo, this transcribed strand combines with natural mRNA produced by the embryo to form duplexes. These duplexes then block either the further transcription of the mRNA or its translation. In this manner, mutant phenotypes may be generated. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. The designation (-) (*i.e.*, "negative") is sometimes used in reference to the antisense strand, with the designation (+) sometimes used in reference to the sense (*i.e.*, "positive") strand.

[0099] The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" or "isolated polynucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids are nucleic acids such as DNA and RNA found in the state they exist in nature. For example, a given DNA sequence (*e.g.*, a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs that encode a multitude of proteins. However, isolated nucleic acid encoding NPHP4 includes, by way of example, such nucleic acid in cells ordinarily expressing NPHP4 where the nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid, oligonucleotide, or polynucleotide may be present in single-stranded or double-stranded form. When an isolated nucleic acid, oligonucleotide or polynucleotide is to be utilized to express a protein, the oligonucleotide or polynucleotide will contain at a minimum the sense or coding strand (*i.e.*, the oligonucleotide or polynucleotide may single-stranded), but may contain both the sense and anti-sense strands (*i.e.*, the oligonucleotide or polynucleotide may be double-stranded).

[0100] As used herein, a "portion of a chromosome" refers to a discrete section of the chromosome. Chromosomes are divided into sites or sections by cytogeneticists as follows: the short (relative to the centromere) arm of a chromosome is termed the "p" arm; the long arm is termed the "q" arm. Each arm is then divided into 2 regions termed region 1 and region 2 (region 1 is closest to the centromere). Each region is further divided into bands. The bands may be further divided into sub-bands. For example, the 11p15.5 portion of human chromosome 11 is the portion located on chromosome 11 (11) on the short arm (p) in the first region (1) in the 5th band (5) in sub-band 5 (.5). A portion of a chromosome may be "altered;" for instance the entire portion may be absent due to a deletion or may be rearranged (*e.g.*, inversions, translocations, expanded or contracted due to changes in repeat regions). In the case of a deletion, an attempt to hybridize (*i.e.*, specifically bind) a probe homologous to a particular portion of a chromosome could result in a negative result (*i.e.*, the probe could not bind to the sample containing genetic material suspected of containing the missing portion of the chromosome). Thus, hybridization of a probe homologous to a particular portion of a chromosome may be used to detect alterations in a portion of a chromosome.

[0101] The term "sequences associated with a chromosome" means preparations of chromosomes (*e.g.*, spreads of metaphase chromosomes), nucleic acid extracted from a sample containing chromosomal DNA (*e.g.*, preparations of genomic DNA); the RNA that is produced by transcription of genes located on a chromosome (*e.g.*, hnRNA and mRNA), and cDNA copies of the RNA transcribed from the DNA located on a chromosome. Sequences associated with a chromosome may be detected by numerous techniques including probing of Southern and Northern blots and *in situ* hybridization to RNA, DNA, or metaphase chromosomes with probes containing sequences homologous to the nucleic acids in the above listed preparations.

[0102] As used herein the term "portion" when in reference to a nucleotide sequence (as in "a portion of a given nucleotide sequence") refers to fragments of that sequence. The fragments may range in size from four nucleotides to the entire nucleotide sequence minus one nucleotide (10 nucleotides, 20, 30, 40, 50, 100, 200, etc.).

[0103] As used herein the term "coding region" when used in reference to structural gene refers to the nucleotide sequences that encode the amino acids found in the nascent polypeptide as a result of translation of a mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet "ATG" that encodes the initiator methionine and on the 3' side by one of the three triplets, which specify stop codons (*i.e.*, TAA, TAG, TGA).

[0104] As used herein, the term "purified" or "to purify" refers to the removal of contaminants from a sample. For example, NPHP4 antibodies are purified by removal of contaminating non-immunoglobulin proteins; they are also purified by the removal of immunoglobulin that does not bind NPHP4. The removal of non-immunoglobulin proteins and/or the removal of immunoglobulins that do not bind NPHP4 results in an increase in the percent of NPHP4-reactive immunoglobulins in the sample. In another example, recombinant NPHP4 polypeptides are expressed in bacterial host cells and the polypeptides are purified by the removal of host cell proteins; the percent of recombinant NPHP4 polypeptides is thereby increased in the sample.

[0105] The term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

[0106] The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule that is expressed from a recombinant DNA molecule.

[0107] The term "native protein" as used herein to indicate that a protein does not contain amino acid residues encoded by vector sequences; that is the native protein contains only those amino acids found in the protein as it occurs in nature. A native protein may be produced by recombinant means or may be isolated from a naturally occurring source.

[0108] As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four consecutive amino acid residues to the entire amino acid sequence minus one amino acid.

[0109] The term "Southern blot," refers to the analysis of DNA on agarose or acrylamide gels to fractionate the DNA according to size followed by transfer of the DNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized DNA is then probed with a labeled probe to detect DNA species complementary to the probe used. The DNA may be cleaved with restriction enzymes prior to electrophoresis. Following electrophoresis, the DNA may be partially depurinated and denatured prior to or during transfer to the solid support. Southern blots are a standard tool of molecular biologists (J. Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, NY, pp 9.31-9.58 [1989]).

[0110] The term "Northern blot," as used herein refers to the analysis of RNA by electrophoresis of RNA on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized RNA is then probed with a labeled probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists (J. Sambrook, *et al., supra*, pp 7.39-7.52 [1989]).

[0111] The term "Western blot" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins, followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabeled antibodies.

[0112] The term "antigenic determinant" as used herein refers to that portion of an antigen that makes contact with a particular antibody (*i.e.*, an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies that bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (*i.e.*, the "immunogen" used to elicit the immune response) for binding to an antibody.

[0113] The term "transgene" as used herein refers to a foreign, heterologous, or autologous gene that is placed into an organism by introducing the gene into newly fertilized eggs or early embryos. The term "foreign gene" refers to any nucleic acid (*e.g.*, gene sequence) that is introduced into the genome of an animal by experimental manipulations and may include gene sequences found in that animal so long as the introduced gene does not reside in the same location as does the naturally-occurring gene. The term "autologous gene" is intended to encompass variants (*e.g.*, polymorphisms or mutants) of the naturally occurring gene. The term transgene thus encompasses the replacement of the naturally occurring gene with a variant form of the gene.

[0114] As used herein, the term "vector" is used in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another. The term "vehicle" is sometimes used interchangeably with "vector."

[0115] The term "expression vector" as used herein refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host organism. Nucleic acid sequences necessary for expression in prokaryotes usually include a promoter, an operator (optional), and a ribosome binding site, often along with other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals.

[0116] As used herein, the term "host cell" refers to any eukaryotic or prokaryotic cell (*e.g.*, bacterial cells such as *E. coli*, yeast cells, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, and insect cells), whether located *in vitro* or *in vivo*. For example, host cells may be located in a transgenic animal.

[0117] The terms "overexpression" and "overexpressing" and grammatical equivalents, are used in reference to levels of mRNA to indicate a level of expression approximately 3-fold higher than that typically observed in a given tissue in a control or non-transgenic animal. Levels of mRNA are measured using any of a number of techniques known to those skilled in the art including, but not limited to Northern blot analysis (*See*, Example 10, for a protocol for performing Northern blot analysis). Appropriate controls are included on the Northern blot to control for differences in the amount of RNA loaded from each tissue analyzed (*e.g.*, the amount of 28S rRNA, an abundant RNA transcript present at essentially the same amount in all tissues, present in each sample can be used as a means of normalizing or standardizing the RAD50 mRNA-specific signal observed on Northern blots). The amount of mRNA present in the band corresponding in size to the correctly spliced NPHP4 transgene RNA is quantified; other minor species of RNA which hybridize to the transgene probe are not considered in the quantification of the expression of the transgenic mRNA.

[0118] The term "transfection" as used herein refers to the introduction of foreign DNA into eukaryotic cells. Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, and biolistics.

[0119] The term "stable transfection" or "stably transfected" refers to the introduction and integration of foreign DNA into the genome of the transfected cell. The term "stable transfectant" refers to a cell that has stably integrated foreign DNA into the genomic DNA.

[0120] The term "transient transfection" or "transiently transfected" refers to the introduction of foreign DNA into a cell where the foreign DNA fails to integrate into the genome of the transfected cell. The foreign DNA persists in the nucleus of the transfected cell for several days. During this time the foreign DNA is subject to the regulatory controls that govern the expression of endogenous genes in the chromosomes. The term "transient transfectant" refers to cells that have taken up foreign DNA but have failed to integrate this DNA.

[0121] The term "calcium phosphate co-precipitation" refers to a technique for the introduction of nucleic acids into a cell. The uptake of nucleic acids by cells is enhanced when the nucleic acid is presented as a calcium phosphate-nucleic acid co-precipitate. The original technique of Graham and van der Eb (Graham and van der Eb, Virol., 52:456 [1973]), has been modified by several groups to optimize conditions for particular types of cells. The art is well aware of these numerous modifications.

[0122] A "composition comprising a given polynucleotide sequence" as used herein refers broadly to any composition containing the given polynucleotide sequence. The composition may comprise an aqueous solution. Compositions comprising polynucleotide sequences encoding NPHP4 (*e.g.*, SEQ ID NO:1) or fragments thereof may be employed as hybridization probes. In this case, the NPHP4 encoding polynucleotide sequences are typically employed in an aqueous solution containing salts (*e.g.*, NaCl), detergents (*e.g.*, SDS), and other components (*e.g.*, Denhardt's solution, dry milk, salmon sperm DNA, etc.).

[0123] The term "test compound" refers to any chemical entity, pharmaceutical, drug, and the like that can be used to treat or prevent a disease, illness, sickness, or disorder of bodily function, or otherwise alter the physiological or cellular status of a sample. Test compounds comprise both known and potential therapeutic compounds. A test compound can be determined to be therapeutic by screening using the screening methods of the present invention. A "known therapeutic compound" refers to a therapeutic compound that has been shown (*e.g.*, through animal trials or prior experience with administration to humans) to be effective in such treatment or prevention.

[0124] The term "sample" as used herein is used in its broadest sense. A sample suspected of containing a human chromosome or sequences associated with a human chromosome may comprise a cell, chromosomes isolated from a cell (*e.g.*, a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern blot analysis), RNA (in solution or bound to a solid support such as for Northern blot analysis), cDNA (in solution or bound to a solid support) and the like. A sample suspected of containing a protein may comprise a cell, a portion of a tissue, an extract containing one or more proteins and the like.

[0125] As used herein, the term "response," when used in reference to an assay, refers to the generation of a detectable signal (*e.g.*, accumulation of reporter protein, increase in ion concentration, accumulation of a detectable chemical product).

[0126] As used herein, the term "membrane receptor protein" refers to membrane spanning proteins that bind a ligand (*e.g.*, a hormone or neurotransmitter). As is known in the art, protein phosphorylation is a common regulatory mechanism used by cells to selectively modify proteins carrying regulatory signals from outside the cell to the nucleus. The proteins that execute these biochemical modifications are a group of enzymes known as protein kinases. They may further be defined by the substrate residue that they target for phosphorylation. One group of protein kinases is the tyrosine kinases (TKs), which selectively phosphorylate a target protein on its tyrosine residues. Some tyrosine kinases are membrane-bound receptors (RTKs), and, upon activation by a ligand, can autophosphorylate as well as modify substrates. The initiation of sequential phosphorylation by ligand stimulation is a paradigm that underlies the action of such effectors as, for example, epidermal growth factor (EGF), insulin, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). The receptors for these ligands are tyrosine kinases and provide the interface between the binding of a ligand (hormone, growth factor) to a target cell and the transmission of a signal into the cell by the activation of one or more biochemical pathways. Ligand binding to a receptor tyrosine kinase activates its intrinsic enzymatic activity. Tyrosine kinases can also be cytoplasmic, non-receptor-type enzymes and act as a downstream component of a signal transduction pathway.

[0127] As used herein, the term "signal transduction protein" refers to proteins that are activated or otherwise affected by ligand binding to a membrane or cytostolic receptor protein or some other stimulus. Examples of signal transduction protein include adenyl cyclase, phospholipase C, and G-proteins. Many membrane receptor proteins are coupled to G-proteins (*i.e.*, G-protein coupled receptors (GPCRs); for a review, *see* Neer, 1995, Cell 80:249-257 [1995]). Typically, GPCRs contain seven transmembrane domains. Putative GPCRs can be identified on the basis of sequence homology to known GPCRs.

[0128] GPCRs mediate signal transduction across a cell membrane upon the binding of a ligand to an extracellular portion of a GPCR. The intracellular portion of a GPCR interacts with a G-protein to modulate signal transduction from outside to inside a cell. A GPCR is therefore said to be "coupled" to a G-protein. G-proteins are composed of three polypeptide subunits: an α subunit, which binds and hydrolyses GTP, and a dimeric $\beta\gamma$ subunit. In the basal, inactive state, the G-protein exists as a heterotrimer of the α and $\beta\gamma$ subunits. When the G-protein is inactive, guanosine diphosphate (GDP) is associated with the α subunit of the G-protein. When a GPCR is bound and activated by a ligand, the GPCR binds to the G-protein heterotrimer and decreases the affinity of the Gα subunit for GDP. In its active state, the G subunit exchanges GDP for guanine triphosphate (GTP) and active Gα subunit disassociates from both the receptor and the dimeric $\beta\gamma$ subunit. The disassociated, active Gα subunit transduces signals to effectors that are "downstream" in the G-protein signaling pathway within the cell. Eventually, the G-protein's endogenous GTPase activity returns active G subunit to its inactive state, in which it is associated with GDP and the dimeric $\beta\gamma$ subunit.

[0129] Numerous members of the heterotrimeric G-protein family have been cloned, including more than 20 genes encoding various Gα subunits. The various G subunits have been categorized into four families, on the basis of amino acid sequences and functional homology. These four families are termed Gα_s, Gα_i, Gα_q, and Gα_{12}. Functionally, these four families differ with respect to the intracellular signaling pathways that they activate and the GPCR to which they couple.

[0130] For example, certain GPCRs normally couple with Gα_s and, through Gα_s, these GPCRs stimulate adenylyl cyclase activity. Other GPCRs normally couple with GGα_q, and through GGα_q, these GPCRs can activate phospholipase C (PLC), such as the β isoform of phospholipase C (*i.e.*, PLCβ, Stermweis and Smrcka, Trends in Biochem. Sci. 17:502-506 [1992]).

[0131] As used herein, the term "reporter gene" refers to a gene encoding a protein that may be assayed. Examples of reporter genes include, but are not limited to, luciferase (*See, e.g.*, deWet *et al.*, Mol. Cell. Biol. 7:725 [1987] and U.S. Pat Nos., 6,074,859; 5,976,796; 5,674,713; and 5,618,682; all of which are incorporated herein by reference), green fluorescent protein (*e.g.*, GenBank Accession Number U43284; a number of GFP variants are commercially available from CLONTECH Laboratories, Palo Alto, CA), chloramphenicol acetyltransferase, β-galactosidase, alkaline phosphatase, and horse radish peroxidase.

[0132] As used herein, the terms "computer memory" and "computer memory device" refer to any storage media readable by a computer processor. Examples of computer memory include, but are not limited to, RAM, ROM, computer chips, digital video disc (DVDs), compact discs (CDs), hard disk drives (HDD), and magnetic tape.

[0133] As used herein, the term "computer readable medium" refers to any device or system for storing and providing information (*e.g.*, data and instructions) to a computer processor. Examples of computer readable media include, but are not limited to, DVDs, CDs, hard disk drives, magnetic tape and servers for streaming media over networks.

[0134] As used herein, the term "entering" as in "entering said genetic variation information into said computer" refers to transferring information to a "computer readable medium." Information may be transferred by any suitable method, including but not limited to, manually (*e.g.*, by typing into a computer) or automated (*e.g.*, transferred from another "computer readable medium" via a "processor").

[0135] As used herein, the terms "processor" and "central processing unit" or "CPU" are used interchangeably and refer to a device that is able to read a program from a computer memory (*e.g.*, ROM or other computer memory) and perform a set of steps according to the program.

[0136] As used herein, the term "computer implemented method" refers to a method utilizing a "CPU" and "computer readable medium."

DETAILED DESCRIPTION OF THE INVENTION

[0137] The present invention relates to Nephronophthisis, in particular to the NPHP4 protein (nephroretinin or nephrocystin-4) and nucleic acids encoding the NPHP4 protein. The present invention also provides assays for the detection of *NPHP4,* and assays for detecting nephroretinin and inversin polymorphisms and mutations associated with disease states.

I. NPHP4 Polynucleotides

[0138] As described above, a new gene associated with NPHP4 kidney disease has been discovered. Accordingly, the present invention provides nucleic acids encoding NPHP4 genes, homologs, variants (*e.g.*, polymorphisms and mutants), including but not limited to, those described in SEQ ID NO: 1. In some embodiments, the present invention provide polynucleotide sequences that are capable of hybridizing to SEQ ID NO: 1 under conditions of low to high stringency as long as the polynucleotide sequence capable of hybridizing encodes a protein that retains a biological activity of the naturally occurring NPHP4. In some embodiments, the protein that retains a biological activity of naturally occurring NPHP4 is 70% homologous to wild-type NPHP4, preferably 80% homologous to wild-type NPHP4, more preferably 90% homologous to wild-type NPHP4, and most preferably 95% homologous to wild-type NPHP4. In preferred embodiments, hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex and confer a defined "stringency" as explained above (*See e.g.*, Wahl, *et al.*, Meth. Enzymol., 152:399-407 [1987], incorporated herein by reference).

[0139] In other embodiments of the present invention, additional alleles of NPHP4 are provided (*e.g.*, as shown in Example 1). In preferred embodiments, alleles result from a polymorphism or mutation (*i.e.*, a change in the nucleic acid sequence) and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes that give rise to alleles are generally ascribed to deletions, additions or substitutions of nucleic acids. Each of these types of changes may occur alone, or in combination with the others, and at the rate of one or more times in a given sequence. Examples of the alleles of the present invention include those encoded by

SEQ ID NOs:1 (wild type) and disease alleles described herein (*e.g.*, SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, and 19).

[0140] In still other embodiments of the present invention, the nucleotide sequences of the present invention may be engineered in order to alter an NPHP4 coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques that are well known in the art (*e.g.*, site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.).

[0141] In some embodiments of the present invention, the polynucleotide sequence of NPHP4 may be extended utilizing the nucleotide sequence (*e.g.*, SEQ ID NO: 1) in various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, it is contemplated that restriction-site polymerase chain reaction (PCR) will find use in the present invention. This is a direct method that uses universal primers to retrieve unknown sequence adjacent to a known locus (Gobinda *et al.*, PCR Methods Applic., 2:318-22 [1993]). First, genomic DNA is amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

[0142] In another embodiment, inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, Nucleic Acids Res., 16:8186 [1988]). The primers may be designed using Oligo 4.0 (National Biosciences Inc, Plymouth Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template. In still other embodiments, walking PCR is utilized. Walking PCR is a method for targeted gene walking that permits retrieval of unknown sequence (Parker *et al.*, Nucleic Acids Res., 19:3055-60 [1991]). The PROMOTERFINDER kit (Clontech) uses PCR, nested primers and special libraries to "walk in" genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

[0143] Preferred libraries for screening for full length cDNAs include mammalian libraries that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred, in that they will contain more sequences that contain the 5' and upstream gene regions. A randomly primed library may be particularly useful in case where an oligo d(T) library does not yield full-length cDNA. Genomic mammalian libraries are useful for obtaining introns and extending 5' sequence.

[0144] In other embodiments of the present invention, variants of the disclosed NPHP4 sequences are provided. In preferred embodiments, variants result from polymorphisms or mutations (*i.e.*, a change in the nucleic acid sequence) and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many variant forms. Common mutational changes that give rise to variants are generally ascribed to deletions, additions or substitutions of nucleic acids. Each of these types of changes may occur alone, or in combination with the others, and at the rate of one or more times in a given sequence.

[0145] It is contemplated that it is possible to modify the structure of a peptide having a function (*e.g.*, NPHP4 function) for such purposes as altering the biological activity (*e.g.*, prevention of cystic kidney disease). Such modified peptides are considered functional equivalents of peptides having an activity of NPHP4 as defined herein. A modified peptide can be produced in which the nucleotide sequence encoding the polypeptide has been altered, such as by substitution, deletion, or addition. In particularly preferred embodiments, these modifications do not significantly reduce the biological activity of the modified NPHP4. In other words, construct "X" can be evaluated in order to determine whether it is a member of the genus of modified or variant NPHP4's of the present invention as defined functionally, rather than structurally. In preferred embodiments, the activity of variant NPHP4 polypeptides is evaluated by methods described herein (*e.g.*, the generation of transgenic animals).

[0146] Moreover, as described above, variant forms of NPHP4 are also contemplated as being equivalent to those peptides and DNA molecules that are set forth in more detail herein. For example, it is contemplated that isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*i.e.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Accordingly, some embodiments of the present invention provide variants of NPHP4 disclosed herein containing conservative replacements. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids can be divided into four families: (1) acidic (aspartate, glutamate); (2) basic (lysine, arginine, histidine); (3) nonpolar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); and (4) uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). Phenylalanine, tryptophan, and tyrosine are.sometimes classified jointly as aromatic amino acids. In similar fashion, the amino acid repertoire can be grouped as (1) acidic (aspartate, glutamate); (2) basic (lysine, arginine, histidine), (3) aliphatic (glycine, alanine, valine, leucine, isoleucine, serine, threonine), with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic (phenylalanine, tyrosine, tryptophan); (5) amide (asparagine, glutamine); and (6) sulfur -containing (cysteine and methionine) (*e.g.*, Stryer ed., *Biochemistry*, pg. 17-21, 2nd ed, WH Freeman and Co., 1981). Whether a change in the amino acid sequence of a peptide results in a functional polypeptide can be readily determined by assessing the ability of the variant peptide to function in a fashion similar to the wild-type protein. Peptides having more than one replacement can readily be tested in the same manner.

[0147] More rarely, a variant includes "nonconservative" changes (*e.g.*, replacement of a glycine with a tryptophan). Analogous minor variations can also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological activity can be found using computer programs (*e.g.*, LASERGENE software, DNASTAR Inc., Madison, Wis.).

[0148] As described in more detail below, variants may be produced by methods such as directed evolution or other techniques for producing combinatorial libraries of variants, described in more detail below. In still other embodiments of the present invention, the nucleotide sequences of the present invention may be engineered in order to alter a NPHP4 coding sequence including, but not limited to, alterations that modify the cloning, processing, localization, secretion, and/or expression of the gene product. For example, mutations may be introduced using techniques that are well known in the art (*e.g.*, site-directed mutagenesis to insert new restriction sites, alter glycosylation patterns, or change codon preference, etc.).

II. NPHP4 Polypeptides

[0149] In other embodiments, the present invention provides NPHP4 polynucleotide sequences that encode NPHP4 polypeptide sequences. NPHP4 polypeptides (*e.g.*, SEQ ID NOs: 2, 6, 8, 10, 12, 14, 16, 18, and 20) are described in Figures 4-13. Other embodiments of the present invention provide fragments, fusion proteins or functional equivalents of these NPHP4 proteins. In some embodiments, the present invention provides truncation mutants of NPHP4 (*e.g.*, SEQ ID NOs: 6, 10, 12, 14, 16, and 20). In still other embodiment of the present invention, nucleic acid sequences corresponding to NPHP4 variants, homologs, and mutants may be used to generate recombinant DNA molecules that direct the expression of the NPHP4 variants, homologs, and mutants in appropriate host cells. In some embodiments of the present invention, the polypeptide may be a naturally purified product, in other embodiments it may be a product of chemical synthetic procedures, and in still other embodiments it may be produced by recombinant techniques using a prokaryotic or eukaryotic host (*e.g.*, by bacterial, yeast, higher plant, insect and mammalian cells in culture). In some embodiments, depending upon the host employed in a recombinant production procedure, the polypeptide of the present invention may be glycosylated or may be non-glycosylated. In other embodiments, the polypeptides of the invention may also include an initial methionine amino acid residue.

[0150] In one embodiment of the present invention, due to the inherent degeneracy of the genetic code, DNA sequences other than the polynucleotide sequences of SEQ ID NO:1 that encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express NPHP4. In general, such polynucleotide sequences hybridize to SEQ ID NO:1 under conditions of high to medium stringency as described above. As will be understood by those of skill in the art, it may be advantageous to produce NPHP4-encoding nucleotide sequences possessing non-naturally occurring codons. Therefore, in some preferred embodiments, codons preferred by a particular prokaryotic or eukaryotic host (Murray *et al.*, Nucl. Acids Res., 17 [1989]) are selected, for example, to increase the rate of NPHP4 expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

1. Vectors for Production of NPHP4

[0151] The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. In some embodiments of the present invention, vectors include, but are not limited to, chromosomal, nonchromosomal and synthetic DNA sequences (*e.g.*, derivatives of SV40, bacterial plasmids, phage DNA; baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies). It is contemplated that any vector may be used as long as it is replicable and viable in the host.

[0152] In particular, some embodiments of the present invention provide recombinant constructs comprising one or more of the sequences as broadly described above (*e.g.*, SEQ ID NOs: 1, 5, 7, 9, 11, 13, 15, 17, and 19). In some embodiments of the present invention, the constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In still other embodiments, the heterologous structural sequence (*e.g.*, SEQ ID NO:1) is assembled in appropriate phase with translation initiation and termination sequences. In preferred embodiments of the present invention, the appropriate DNA sequence is inserted into the vector using any of a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art.

[0153] Large numbers of suitable vectors are known to those of skill in the art, and are commercially available. Such vectors include, but are not limited to, the following vectors: 1) Bacterial -- pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, phagescript, psiX174, pbluescript SK, pBSKS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); 2) Eukaryotic -- pWLNEO, pSV2CAT, pOG44, PXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia); and 3) Baculovirus – pPbac and pMbac (Stratagene). Any other plasmid or vector may be used as long as they are replicable and viable in the host. In some preferred embodiments of the present invention, mammalian expression vectors comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. In other embodiments, DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

[0154] In certain embodiments of the present invention, the DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. Promoters useful in the present invention include, but are not limited to, the LTR or SV40 promoter, the *E. coli lac* or *trp*, the phage lambda P_L and P_R, T3 and T7 promoters, and the cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, and mouse metallothionein-I promoters and other promoters known to control expression of gene in prokaryotic or eukaryotic cells or their viruses. In other embodiments of the present invention, recombinant expression vectors include origins of replication and selectable markers permitting transformation of the host cell (*e.g.*, dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or tetracycline or ampicillin resistance in *E. coli*).

[0155] In some embodiments of the present invention, transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are *cis*-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Enhancers useful in the present invention include, but are not limited to, the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[0156] In other embodiments, the expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. In still other embodiments of the present invention, the vector may also include appropriate sequences for amplifying expression.

2. Host Cells for Production of NPHP4

[0157] In a further embodiment, the present invention provides host cells containing the above-described constructs. In some embodiments of the present invention, the host cell is a higher eukaryotic cell (*e.g.*, a mammalian or insect cell). In other embodiments of the present invention, the host cell is a lower eukaryotic cell (*e.g.*, a yeast cell). In still other embodiments of the present invention, the host cell can be a prokaryotic cell (*e.g.*, a bacterial cell). Specific examples of host cells include, but are not limited to, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, as well as *Saccharomycees cerivisiae*, *Schizosaccharomycees pombe*, *Drosophila* S2 cells, *Spodoptera* Sf9 cells, Chinese hamster ovary (CHO) cells, COS-7 lines of monkey kidney fibroblasts, (Gluzman, Cell 23:175 [1981]), C127, 3T3, 293, 293T, HeLa and BHK cell lines.

[0158] The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. In some embodiments, introduction of the construct into the host cell can be accomplished by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (*See e.g.*, Davis *et al.*, Basic Methods in Molecular Biology, [1986]). Alternatively, in some embodiments of the present invention, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

[0159] Proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., [1989].

[0160] In some embodiments of the present invention, following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period. In other embodiments of the present invention, cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. In still other embodiments of the present invention, microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

3. Purification of NPHP4

[0161] The present invention also provides methods for recovering and purifying NPHP4 from recombinant cell cultures including, but not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. In other embodiments of the present invention, protein-refolding steps can be used as necessary, in completing configuration of the mature protein. In still other embodiments of the present invention, high performance liquid chromatography (HPLC) can be employed for final purification steps.

[0162] The present invention further provides polynucleotides having the coding sequence (*e.g.*, SEQ ID NO: 1) fused in frame to a marker sequence that allows for purification of the polypeptide of the present invention. A non-limiting example of a marker sequence is a hexahistidine tag which may be supplied by a vector, preferably a pQE-9 vector, which provides for purification of the polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host (*e.g.*, COS-7 cells) is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, Cell, 37:767 [1984]).

4. Truncation Mutants of NPHP4

[0163] In addition, the present invention provides fragments of NPHP4 (*i.e.*, truncation mutants, *e.g.*, SEQ ID NOs: 6, 10, 12, 14, 16, and 20). As described above, truncations of NPHP4 were found in families with NPHP type 4 disease. In some embodiments of the present invention, when expression of a portion of the NPHP4 protein is desired, it may be necessary to add a start codon (ATG) to the oligonucleotide fragment containing the desired sequence to be expressed. It is well known in the art that a methionine at the N-terminal position can be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP). MAP has been cloned from *E. coli* (Ben-Bassat *et al.*, J. Bacteriol., 169:751 [1987]) and *Salmonella typhimurium* and its *in vitro* activity has been demonstrated on recombinant proteins (Miller *et al.*, Proc. Natl. Acad. Sci. USA 84:2718 [1990]). Therefore, removal of an N-terminal methionine, if desired, can be achieved either *in vivo* by expressing such recombinant polypeptides in a host which produces MAP (*e.g.*, *E. coli* or CM89 or *S. cerivisiae*), or *in vitro* by use of purified MAP.

5. Fusion Proteins Containing NPHP4

[0164] The present invention also provides fusion proteins incorporating all or part of NPHP4. Accordingly, in some embodiments of the present invention, the coding sequences for the polypeptide can be incorporated as a part of a fusion gene including a nucleotide sequence encoding a different polypeptide. It is contemplated that this type of expression system will find use under conditions where it is desirable to produce an immunogenic fragment of a NPHP4 protein. In some embodiments of the present invention, the VP6 capsid protein of rotavirus is used as an immunologic carrier protein for portions of the NPHP4 polypeptide, either in the monomeric form or in the form of a viral particle. In other embodiments of the present invention, the nucleic acid sequences corresponding to the portion of NPHP4 against which antibodies are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia virus structural protein to produce a set of recombinant viruses expressing fusion proteins comprising a portion of NPHP4 as part of the virion. It has been demonstrated with the use of immunogenic fusion proteins utilizing the hepatitis B surface antigen fusion proteins that recombinant hepatitis B virions can be utilized in this role as well. Similarly, in other embodiments of the present invention, chimeric constructs coding for fusion proteins containing a portion of NPHP4 and the poliovirus capsid protein are created to enhance immunogenicity of the set of polypeptide antigens (*See e.g.*, EP Publication No. 025949; and Evans *et al.*, Nature 339:385 [1989]; Huang *et al.*, J. Virol., 62:3855 [1988]; and Schlienger *et al.*, J. Virol., 66:2 [1992]).

[0165] In still other embodiments of the present invention, the multiple antigen peptide system for peptide-based immunization can be utilized. In this system, a desired portion of NPHP4 is obtained directly from organo-chemical synthesis of the peptide onto an oligomeric branching lysine core (*see e.g.*, Posnett *et al.*, J. Biol. Chem., 263:1719 [1988]; and Nardelli *et al.*, J. Immunol., 148:914 [1992]). In other embodiments of the present invention, antigenic determinants of the NPHP4 proteins can also be expressed and presented by bacterial cells.

[0166] In addition to utilizing fusion proteins to enhance immunogenicity, it is widely appreciated that fusion proteins can also facilitate the expression of proteins, such as the NPHP4 protein of the present invention. Accordingly, in some embodiments of the present invention, NPHP4 can be generated as a glutathione-S-transferase (*i.e.*, GST fusion protein). It is contemplated that such GST fusion proteins will enable easy purification of NPHP4, such as by the use of glutathione-derivatized matrices (*See e.g.*, Ausabel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY [1991]). In another embodiment of the present invention, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of NPHP4, can allow purification of the expressed NPHP4 fusion protein by affinity chromatography using a Ni^{2+} metal resin. In still another embodiment of the present invention, the purification leader sequence can then be subsequently removed by treatment with enterokinase (*See e.g.*, Hochuli *et al.*, J. Chromatogr., 411:177 [1987]; and Janknecht *et al.*, Proc. Natl. Acad. Sci. USA 88:8972).

[0167] Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment of the present invention, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, in other embodiments of the present invention, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence (*See e.g.*, Current Protocols in Molecular Biology, *supra*).

6. Variants of NPHP4

[0168] Still other embodiments of the present invention provide mutant or variant forms of NPHP4 (*i.e.*, muteins). It is possible to modify the structure of a peptide having an activity of NPHP4 for such purposes as enhancing therapeutic or prophylactic efficacy, or stability (*e.g.*, *ex vivo* shelf life, and/or resistance to proteolytic degradation *in vivo*). Such modified peptides are considered functional equivalents of peptides having an activity of the subject NPHP4 proteins as defined herein. A modified peptide can be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion, or addition.

[0169] Moreover, as described above, variant forms (*e.g.*, mutants or polymorphic sequences) of the subject NPHP4 proteins are also contemplated as being equivalent to those peptides and DNA molecules that are set forth in more detail. For example, as described above, the present invention encompasses mutant and variant proteins that contain conservative or non-conservative amino acid substitutions.

[0170] This invention further contemplates a method of generating sets of combinatorial mutants of the present NPHP4 proteins, as well as truncation mutants, and is especially useful for identifying potential variant sequences (*i.e.*, mutants or polymorphic sequences) that are involved in kidney disease or resistance to kidney disease. The purpose of screening such combinatorial libraries is to generate, for example, novel NPHP4 variants that can act as either agonists or antagonists, or alternatively, possess novel activities all together.

[0171] Therefore, in some embodiments of the present invention, NPHP4 variants are engineered by the present method to provide altered (*e.g.*, increased or decreased) biological activity. In other embodiments of the present invention, combinatorially-derived variants are generated which have a selective potency relative to a naturally occurring NPHP4. Such proteins, when expressed from recombinant DNA constructs, can be used in gene therapy protocols.

[0172] Still other embodiments of the present invention provide NPHP4 variants that have intracellular half-lives dramatically different than the corresponding wild-type protein. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular process that result in destruction of, or otherwise inactivate NPHP4. Such variants, and the genes which encode them, can be utilized to alter the location of NPHP4 expression by modulating the half-life of the protein. For instance, a short half-life can give rise to more transient NPHP4 biological effects and, when part of an inducible expression system, can allow tighter control of NPHP4 levels within the cell. As above, such proteins, and particularly their recombinant nucleic acid constructs, can be used in gene therapy protocols.

[0173] In still other embodiments of the present invention, NPHP4 variants are generated by the combinatorial approach to act as antagonists, in that they are able to interfere with the ability of the corresponding wild-type protein to regulate cell function.

[0174] In some embodiments of the combinatorial mutagenesis approach of the present invention, the amino acid sequences for a population of NPHP4 homologs, variants or other related proteins are aligned, preferably to promote the highest homology possible. Such a population of variants can include, for example, NPHP4 homologs from one or more species, or NPHP4 variants from the same species but which differ due to mutation or polymorphisms.

Amino acids that appear at each position of the aligned sequences are selected to create a degenerate set of combinatorial sequences.

[0175] In a preferred embodiment of the present invention, the combinatorial NPHP4 library is produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential NPHP4 protein sequences. For example, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential NPHP4 sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of NPHP4 sequences therein.

[0176] There are many ways by which the library of potential NPHP4 homologs and variants can be generated from a degenerate oligonucleotide sequence. In some embodiments, chemical synthesis of a degenerate gene sequence is carried out in an automatic DNA synthesizer, and the synthetic genes are ligated into an appropriate gene for expression. The purpose of a degenerate set of genes is to provide, in one mixture, all of the sequences encoding the desired set of potential NPHP4 sequences. The synthesis of degenerate oligonucleotides is well known in the art (*See e.g.*, Narang, Tetrahedron Lett., 39:39 [1983]; Itakura *et al.*, Recombinant DNA, *in* Walton (ed.), *Proceedings of the 3rd Cleveland Symposium on Macromolecules,* Elsevier, Amsterdam, pp 273-289 [1981]; Itakura *et al.*, Annu. Rev. Biochem., 53:323 [1984]; Itakura *et al.*, Science 198:1056 [1984]; Ike *et al.*, Nucl. Acid Res., 11:477 [1983]). Such techniques have been employed in the directed evolution of other proteins (*See e.g.*, Scott *et al.*, Science 249:386 [1980]; Roberts *et al.*, Proc. Natl. Acad. Sci. USA 89:2429 [1992]; Devlin *et al.*, Science 249: 404 [1990]; Cwirla *et al.*, Proc. Natl. Acad. Sci. USA 87: 6378 [1990]; each of which is herein incorporated by reference; as well as U.S. Pat. Nos. 5,223,409, 5,198,346, and 5,096,815; each of which is incorporated herein by reference).

[0177] It is contemplated that the NPHP4 nucleic acids (*e.g.*, SEQ ID NO:1, and fragments and variants thereof) can be utilized as starting nucleic acids for directed evolution. These techniques can be utilized to develop NPHP4 variants having desirable properties such as increased or decreased biological activity.

[0178] In some embodiments, artificial evolution is performed by random mutagenesis (*e.g.*, by utilizing error-prone PCR to introduce random mutations into a given coding sequence). This method requires that the frequency of mutation be finely tuned. As a general rule, beneficial mutations are rare, while deleterious mutations are common. This is because the combination of a deleterious mutation and a beneficial mutation often results in an inactive enzyme. The ideal number of base substitutions for targeted gene is usually between 1.5 and 5 (Moore and Arnold, Nat. Biotech., 14, 458 [1996]; Leung *et al.*, Technique, 1:11 [1989]; Eckert and Kunkel, PCR Methods Appl., 1:17-24 [1991]; Caldwell and Joyce, PCR Methods Appl., 2:28 [1992]; and Zhao and Arnold, Nuc. Acids. Res., 25:1307 [1997]). After mutagenesis, the resulting clones are selected for desirable activity (*e.g.*, screened for NPHP4 activity). Successive rounds of mutagenesis and selection are often necessary to develop enzymes with desirable properties. It should be noted that only the useful mutations are carried over to the next round of mutagenesis.

[0179] In other embodiments of the present invention, the polynucleotides of the present invention are used in gene shuffling or sexual PCR procedures (*e.g.*, Smith, Nature, 370:324 [1994]; U.S. Pat. Nos. 5,837,458; 5,830,721; 5,811,238; 5,733,731; all of which are herein incorporated by reference). Gene shuffling involves random fragmentation of several mutant DNAs followed by their reassembly by PCR into full length molecules. Examples of various gene shuffling procedures include, but are not limited to, assembly following DNase treatment, the staggered extension process (STEP), and random priming *in vitro* recombination. In the DNase mediated method, DNA segments isolated from a pool of positive mutants are cleaved into random fragments with DNaseI and subjected to multiple rounds of PCR with no added primer. The lengths of random fragments approach that of the uncleaved segment as the PCR cycles proceed, resulting in mutations in present in different clones becoming mixed and accumulating in some of the resulting sequences. Multiple cycles of selection and shuffling have led to the functional enhancement of several enzymes (Stemmer, Nature, 370:398 [1994]; Stemmer, Proc. Natl. Acad. Sci. USA, 91:10747 [1994]; Crameri *et al.*, Nat. Biotech., 14:315 [1996]; Zhang *et al.*, Proc. Natl. Acad. Sci. USA, 94:4504 [1997]; and Crameri *et al.*, Nat. Biotech., 15:436 [1997]). Variants produced by directed evolution can be screened for NPHP4 activity by the methods described herein.

[0180] A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations, and for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis or recombination of NPHP4 homologs or variants. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected.

7. Chemical Synthesis of NPHP4

[0181] In an alternate embodiment of the invention, the coding sequence of NPHP4 is synthesized, whole or in part, using chemical methods well known in the art (*See e.g.*, Caruthers *et al.*, Nucl. Acids Res. Symp. Ser., 7:215 [1980]; Crea and Horn, Nucl. Acids Res., 9:2331 [1980]; Matteucci and Caruthers, Tetrahedron Lett., 21:719 [1980]; and Chow and Kempe, Nucl. Acids Res., 9:2807 [1981]). In other embodiments of the present invention, the protein itself is produced using chemical methods to synthesize either an entire NPHP4 amino acid sequence or a portion thereof. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (*See e.g.*, Creighton, *Proteins Structures And Molecular Principles*, W H Freeman and Co, New York N.Y. [1983]). In other embodiments of the present invention, the composition of the synthetic peptides is confirmed by amino acid analysis or sequencing (*See e.g.*, Creighton, *supra*).

[0182] Direct peptide synthesis can be performed using various solid-phase techniques (Roberge *et al.*, Science 269:202 [1995]) and automated synthesis may be achieved, for example, using ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer. Additionally, the amino acid sequence of NPHP4, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with other sequences to produce a variant polypeptide.

III. Detection of NPHP4 and Inversin Alleles

[0183] In some embodiments, the present invention provides methods of detecting the presence of wild type or variant (*e.g.*, mutant or polymorphic) NPHP4 nucleic acids or polypeptides and inversin nucleic acids and polypeptides. The detection of mutant NPHP4 polypeptides and inversin polypeptides finds use in the diagnosis of disease (*e.g.*, NPHP type 4 or type 2 disease).

A. NPHP4 and Inversin Alleles

[0184] In some embodiments, the present invention includes alleles of NPHP4 and inversin that increase a patient's susceptibility to NPHP type 4 or type 2 kidney disease (*e.g.*, including, but not limited to, SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19, 23, 25, 27, 29, 33, 35, 37, and 39; also see Example 1 and Example 2). However, the present invention is not limited to the mutations described in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19, 23, 25, 27, 29, 33, 35, 37, and 39. Any mutation that results in the undesired phenotype (*e.g.*, kidney disease) is within the scope of the present invention.

B. Detection of NPHP4 and Inversin Alleles

[0185] Accordingly, the present invention provides methods for determining whether a patient has an increased susceptibility NPHP type 4 or type 2 kidney disease by determining whether the individual has a variant NPHP4 allele or inversin allele, respectively. In other embodiments, the present invention provides methods for providing a prognosis of increased risk for kidney disease to an individual based on the presence or absence of one or more variant alleles of NPHP4 or inversin. In preferred embodiments, the variation causes a truncation of the NPHP4 protein or inversin protein.

[0186] A number of methods are available for analysis of variant (*e.g.*, mutant or polymorphic) nucleic acid sequences. Assays for detection variants (*e.g.*, polymorphisms or mutations) fall into several categories, including, but not limited to direct sequencing assays, fragment polymorphism assays, hybridization assays, and computer based data analysis. Protocols and commercially available kits or services for performing multiple variations of these assays are available. In some embodiments, assays are performed in combination or in hybrid (*e.g.*, different reagents or technologies from several assays are combined to yield one assay). The following assays are useful in the present invention.

1. Direct sequencing Assays

[0187] In some embodiments of the present invention, variant sequences are detected using a direct sequencing technique. In these assays, DNA samples are first isolated from a subject using any suitable method. In some embodiments, the region of interest is cloned into a suitable vector and amplified by growth in a host cell (*e.g.*, a bacteria). In other embodiments, DNA in the region of interest is amplified using PCR.

[0188] Following amplification, DNA in the region of interest (*e.g.*, the region containing the SNP or mutation of interest) is sequenced using any suitable method, including but not limited to manual sequencing using radioactive marker nucleotides, or automated sequencing. The results of the sequencing are displayed using any suitable method. The sequence is examined and the presence or absence of a given SNP or mutation is determined.

2. PCR Assay

[0189] In some embodiments of the present invention, variant sequences are detected using a PCR-based assay. In some embodiments, the PCR assay comprises the use of oligonucleotide primers that hybridize only to the variant or wild type allele of NPHP4 or inversin (*e.g.*, to the region of polymorphism or mutation). Both sets of primers are used to amplify a sample of DNA. If only the mutant primers result in a PCR product, then the patient has the mutant NPHP4 allele. If only the wild-type primers result in a PCR product, then the patient has the wild type allele of NPHP4 or inversin.

3. Mutational detection by dHPLC

[0190] In some embodiments of the present invention, variant sequences are detected using a PCR-based assay with consecutive detection of nucleotide variants by dHPLC (denaturing high performance liquid chromatography). Exemplary systems and
Methods for dHPLC include, but are not limited to, WAVE (Transgenomic, Inc; Omaha, NE) or VARIAN equipment (Palo Alto, CA).

4. Fragment Length Polymorphism Assays

[0191] In some embodiments of the present invention, variant sequences are detected using a fragment length polymorphism assay. In a fragment length polymorphism assay, a unique DNA banding pattern based on cleaving the DNA at a series of positions is generated using an enzyme (*e.g.*, a restriction enzyme or a CLEAVASE I [Third Wave Technologies, Madison, WI] enzyme). DNA fragments from a sample containing a SNP or a mutation will have a different banding pattern than wild type.

a. RFLP Assay

[0192] In some embodiments of the present invention, variant sequences are detected using a restriction fragment length polymorphism assay (RFLP). The region of interest is first isolated using PCR. The PCR products are then cleaved with restriction enzymes known to give a unique length fragment for a given polymorphism. The restriction-enzyme digested PCR products are separated by agarose gel electrophoresis and visualized by ethidium bromide staining. The length of the fragments is compared to molecular weight markers and fragments generated from wild-type and mutant controls.

b. CFLP Assay

[0193] In other embodiments, variant sequences are detected using a CLEAVASE fragment length polymorphism assay (CFLP; Third Wave Technologies, Madison, WI; *See e.g.*, U.S. Patent Nos. 5,843,654; 5,843,669; 5,719,208; and 5,888,780; each of which is herein incorporated by reference). This assay is based on the observation that when single strands of DNA fold on themselves, they assume higher order structures that are highly individual to the precise sequence of the DNA molecule. These secondary structures involve partially duplexed regions of DNA such that single stranded regions are juxtaposed with double stranded DNA hairpins. The CLEAVASE I enzyme, is a structure-specific, thermostable nuclease that recognizes and cleaves the junctions between these single-stranded and double-stranded regions.

[0194] The region of interest is first isolated, for example, using PCR. Then, DNA strands are separated by heating. Next, the reactions are cooled to allow intrastrand secondary structure to form. The PCR products are then treated with the CLEAVASE I enzyme to generate a series of fragments that are unique to a given SNP or mutation. The CLEAVASE enzyme treated PCR products are separated and detected (*e.g.*, by agarose gel electrophoresis) and visualized (*e.g.*, by ethidium bromide staining). The length of the fragments is compared to molecular weight markers and fragments generated from wild-type and mutant controls.

5. Hybridization Assays

[0195] In preferred embodiments of the present invention, variant sequences are detected a hybridization assay. In a hybridization assay, the presence of absence of a given SNP or mutation is determined based on the ability of the DNA from the sample to hybridize to a complementary DNA molecule (*e.g.*, a oligonucleotide probe). A variety of hybridization assays using a variety of technologies for hybridization and detection are available. A description of a selection of assays is provided below.

a. Direct Detection of Hybridization

[0196] In some embodiments, hybridization of a probe to the sequence of interest (*e.g.*, a SNP or mutation) is detected directly by visualizing a bound probe (*e.g.*, a Northern or Southern assay; *See e.g.*, Ausabel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY [1991]). In a these assays, genomic DNA (Southern) or RNA (Northern) is isolated from a subject. The DNA or RNA is then cleaved with a series of restriction enzymes that cleave infrequently in the genome and not near any of the markers being assayed. The DNA or RNA is then separated (*e.g.*, on an agarose gel) and transferred to a membrane. A labeled (*e.g.*, by incorporating a radionucleotide) probe or probes specific for the SNP or mutation being detected is allowed to contact the membrane under a condition or low, medium, or high stringency conditions. Unbound probe is removed and the presence of binding is detected by visualizing the labeled probe.

b. Detection of Hybridization Using "DNA Chip" Assays

[0197] In some embodiments of the present invention, variant sequences are detected using a DNA chip hybridization assay. In this assay, a series of oligonucleotide probes are affixed to a solid support. The oligonucleotide probes are designed to be unique to a given SNP or mutation. The DNA sample of interest is contacted with the DNA "chip" and hybridization is detected.

[0198] In some embodiments, the DNA chip assay is a GeneChip (Affymetrix, Santa Clara, CA; *See e.g.*, U.S. Patent Nos. 6,045,996; 5,925,525; and 5,858,659; each of which is herein incorporated by reference) assay. The GeneChip technology uses miniaturized, high-density arrays of oligonucleotide probes affixed to a "chip." Probe arrays are manufactured by Affymetrix's light-directed chemical synthesis process, which combines solid-phase chemical synthesis with photolithographic fabrication techniques employed in the semiconductor industry. Using a series of photolithographic masks to define chip exposure sites, followed by specific chemical synthesis steps, the process constructs high-density arrays of oligonucleotides, with each probe in a predefined position in the array. Multiple probe arrays are synthesized simultaneously on a large glass wafer. The wafers are then diced, and individual probe arrays are packaged in injection-molded plastic cartridges, which protect them from the environment and serve as chambers for hybridization.

[0199] The nucleic acid to be analyzed is isolated, amplified by PCR, and labeled with a fluorescent reporter group. The labeled DNA is then incubated with the array using a fluidics station. The array is then inserted into the scanner, where patterns of hybridization are detected. The hybridization data are collected as light emitted from the fluorescent reporter groups already incorporated into the target, which is bound to the probe array. Probes that perfectly match the target generally produce stronger signals than those that have mismatches. Since the sequence and position of each probe on the array are known, by complementarity, the identity of the target nucleic acid applied to the probe array can be determined.

[0200] In other embodiments, a DNA microchip containing electronically captured probes (Nanogen, San Diego, CA) is utilized (*See e.g.*, U.S. Patent Nos. 6,017,696; 6,068,818; and 6,051,380; each of which are herein incorporated by reference). Through the use of microelectronics, Nanogen's technology enables the active movement and concentration of charged molecules to and from designated test sites on its semiconductor microchip. DNA capture probes unique to a given SNP or mutation are electronically placed at, or "addressed" to, specific sites on the microchip. Since DNA has a strong negative charge, it can be electronically moved to an area of positive charge.

[0201] First, a test site or a row of test sites on the microchip is electronically activated with a positive charge. Next, a solution containing the DNA probes is introduced onto the microchip. The negatively charged probes rapidly move to the positively charged sites, where they concentrate and are chemically bound to a site on the microchip. The microchip is then washed and another solution of distinct DNA probes is added until the array of specifically bound DNA probes is complete.

[0202] A test sample is then analyzed for the presence of target DNA molecules by determining which of the DNA capture probes hybridize, with complementary DNA in the test sample (*e.g.*, a PCR amplified gene of interest). An electronic charge is also used to move and concentrate target molecules to one or more test sites on the microchip. The electronic concentration of sample DNA at each test site promotes rapid hybridization of sample DNA with complementary capture probes (hybridization may occur in minutes). To remove any unbound or nonspecifically bound DNA from each site, the polarity or charge of the site is reversed to negative, thereby forcing any unbound or nonspecifically bound DNA back into solution away from the capture probes. A laser-based fluorescence scanner is used to detect binding,

[0203] In still further embodiments, an array technology based upon the segregation of fluids on a flat surface (chip) by differences in surface tension (ProtoGene, Palo Alto, CA) is utilized (*See e.g.*, U.S. Patent Nos. 6,001,311; 5,985,551; and 5,474,796; each of which is herein incorporated by reference). Protogene's technology is based on the fact that fluids can be segregated on a flat surface by differences in surface tension that have been imparted by chemical coatings. Once so segregated, oligonucleotide probes are synthesized directly on the chip by ink-jet printing of reagents. The array with its reaction sites defined by surface tension is mounted on a X/Y translation stage under a set of four piezoelectric nozzles, one for each of the four standard DNA bases. The translation stage moves along each of the rows of the array and the appropriate reagent is delivered to each of the reaction site. For example, the A amidite is delivered only to the sites where amidite A is to be coupled during that synthesis step and so on. Common reagents and washes are delivered by flooding the entire surface and then removing them by spinning.

[0204] DNA probes unique for the SNP or mutation of interest are affixed to the chip using Protogene's technology. The chip is then contacted with the PCR-amplified genes of interest. Following hybridization, unbound DNA is removed and hybridization is detected using any suitable method (*e.g.*, by fluorescence de-quenching of an incorporated fluorescent group).

[0205] In yet other embodiments, a "bead array" is used for the detection of polymorphisms (Illumina, San Diego, CA; *See e.g.*, PCT Publications WO 99/67641 and WO 00/39587, each of which is herein incorporated by reference). Illumina uses a BEAD ARRAY technology that combines fiber optic bundles and beads that self-assemble into an array. Each fiber optic bundle contains thousands to millions of individual fibers depending on the diameter of the bundle. The beads are coated with an oligonucleotide specific for the detection of a given SNP or mutation. Batches of beads are combined to form a pool specific to the array. To perform an assay, the BEAD ARRAY is contacted with a prepared subject sample (*e.g.*, DNA). Hybridization is detected using any suitable method.

c. Enzymatic Detection of Hybridization

[0206] In some embodiments of the present invention, hybridization is detected by enzymatic cleavage of specific structures (INVADER assay, Third Wave Technologies; *See e.g.*, U.S. Patent Nos. 5,846,717, 6,090,543; 6,001,567; 5,985,557; and 5,994,069; each of which is herein incorporated by reference). The INVADER assay detects specific DNA and RNA sequences by using structure-specific enzymes to cleave a complex formed by the hybridization of overlapping oligonucleotide probes. Elevated temperature and an excess of one of the probes enable multiple probes to be cleaved for each target sequence present without temperature cycling. These cleaved probes then direct cleavage of a second labeled probe. The secondary probe oligonucleotide can be 5'-end labeled with fluorescein that is quenched by an internal dye. Upon cleavage, the de-quenched fluorescein labeled product may be detected using a standard fluorescence plate reader.

[0207] The INVADER assay detects specific mutations and SNPs in unamplified genomic DNA. The isolated DNA sample is contacted with the first probe specific either for a SNP/mutation or wild type sequence and allowed to hybridize. Then a secondary probe, specific to the first probe, and containing the fluorescein label, is hybridized and the enzyme is added. Binding is detected by using a fluorescent plate reader and comparing the signal of the test sample to known positive and negative controls.

[0208] In some embodiments, hybridization of a bound probe is detected using a TaqMan assay (PE Biosystems, Foster City, CA; *See e.g.*, U.S. Patent Nos. 5,962,233 and 5,538,848, each of which is herein incorporated by reference). The assay is performed during a PCR reaction. The TaqMan assay exploits the 5'-3' exonuclease activity of the AMPLITAQ GOLD DNA polymerase. A probe, specific for a given allele or mutation, is included in the PCR reaction. The probe consists of an oligonucleotide with a 5'-reporter dye (*e.g.*, a fluorescent dye) and a 3'-quencher dye. During PCR, if the probe is bound to its target, the 5'-3' nucleolytic activity of the AMPLITAQ GOLD polymerase cleaves the probe between the reporter and the quencher dye. The separation of the reporter dye from the quencher dye results in an increase of fluorescence. The signal accumulates with each cycle of PCR and can be monitored with a fluorimeter.

[0209] In still further embodiments, polymorphisms are detected using the SNP-IT primer extension assay (Orchid Biosciences, Princeton, NJ; *See e.g.*, U.S. Patent Nos. 5,952,174 and 5,919,626, each of which is herein incorporated by reference). In this assay, SNPs are identified by using a specially synthesized DNA primer and a DNA polymerase to selectively extend the DNA chain by one base at the suspected SNP location. DNA in the region of interest is amplified and denatured. Polymerase reactions are then performed using miniaturized systems called microfluidics. Detection is accomplished by adding a label to the nucleotide suspected of being at the SNP or mutation location. Incorporation of the label into the DNA can be detected by any suitable method (*e.g.*, if the nucleotide contains a biotin label, detection is via a fluorescently labeled antibody specific for biotin).

6. Mass Spectroscopy Assay

[0210] In some embodiments, a MassARRAY system (Sequenom, San Diego, CA.) is used to detect variant sequences (*See e.g.*, U.S. Patent Nos. 6,043,031; 5,777,324; and 5,605,798; each of which is herein incorporated by reference). DNA is isolated from blood samples using standard procedures. Next, specific DNA regions containing the mutation or SNP of interest, about 200 base pairs in length, are amplified by PCR. The amplified fragments are then attached by one strand to a solid surface and the non-immobilized strands are removed by standard denaturation and washing. The remaining immobilized single strand then serves as a template for automated enzymatic reactions that produce genotype specific diagnostic products.

[0211] Very small quantities of the enzymatic products, typically five to ten nanoliters, are then transferred to a SpectroCHIP array for subsequent automated analysis with the SpectroREADER mass spectrometer. Each spot is preloaded with light absorbing crystals that form a matrix with the dispensed diagnostic product. The MassARRAY system uses MALDI-TOF (Matrix Assisted Laser Desorption Ionization - Time of Flight) mass spectrometry. In a process known as desorption, the matrix is hit with a pulse from a laser beam. Energy from the laser beam is transferred to the matrix and it is vaporized resulting in a small amount of the diagnostic product being expelled into a flight tube. As the diagnostic product is charged when an electrical field pulse is subsequently applied to the tube they are launched down the flight tube towards a detector. The time between application of the electrical field pulse and collision of the diagnostic product with the detector is referred to as the time of flight. This is a very precise measure of the product's molecular weight, as a molecule's mass correlates directly with time of flight with smaller molecules flying faster than larger molecules. The entire assay is completed in less than one thousandth of a second, enabling samples to be analyzed in a total of 3-5 second including repetitive data collection. The SpectroTYPER software then calculates, records, compares and reports the genotypes at the rate of three seconds per sample.

7. Detection of Variant NPHP4 and Inversin Proteins

[0212] In other embodiments, variant (*e.g.*, truncated) NPHP4 polypeptides and inversin polypeptides are detected (*e.g.*, including, but not limited to, those described in SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, 20, 24, 26, 28, 30, 34, 36, 38 and 40). Any suitable method may be used to detect truncated or mutant NPHP4 polypeptides including, but not limited to, those described below.

a) Cell Free Translation

[0213] For example, in some embodiments, cell-free translation methods from Ambergen, Inc. (Boston, MA) are utilized. Ambergen, Inc. has developed a method for the labeling, detection, quantitation, analysis and isolation of nascent proteins produced in a cell-free or cellular translation system without the use of radioactive amino acids or other radioactive labels. Markers are aminoacylated to tRNA molecules. Potential markers include native amino acids, non-native amino acids, amino acid analogs or derivatives, or chemical moieties. These markers are introduced into nascent proteins from the resulting misaminoacylated tRNAs during the translation process.

[0214] One application of Ambergen's protein labeling technology is the gel free truncation test (GFTT) assay (See *e.g.*, U.S. Patent 6,303,337, herein incorporated by reference). In some embodiments, this assay is used to screen for truncation mutations in a TSC1 or TSC2 protein. In the GFTT assay, a marker (*e.g.*, a fluorophore) is introduced to the nascent protein during translation near the N-terminus of the protein. A second and different marker (*e.g.*, a fluorophore with a different emission wavelength) is introduced to the nascent protein near the C-terminus of the protein. The protein is then separated from the translation system and the signal from the markers is measured. A comparison of the measurements from the N and C terminal signals provides information on the fraction of the molecules with C-terminal truncation (*i.e.*, if the normalized signal from the C-terminal marker is 50% of the signal from the N-terminal marker, 50% of the molecules have a C-terminal truncation).

b) Antibody Binding

[0215] In still further embodiments of the present invention, antibodies (See below for antibody production) are used to determine if an individual contains an allele encoding a variant NPHP4 or inversin gene. In preferred embodiments, antibodies are utilized that discriminate between variant (*i.e.*, truncated proteins); and wild-type proteins (SEQ ID NOs: 2 and 22). In some particularly preferred embodiments, the antibodies are directed to the C-terminus of NPHP4 or inversin. Proteins that are recognized by the N-terminal, but not the C-terminal antibody are truncated. In some embodiments, quantitative immunoassays are used to determine the ratios of C-terminal to N-terminal antibody binding. In other embodiments, identification of variants of NPHP4 or inversin is accomplished through the use of antibodies that differentially bind to wild type or variant forms of NPHP4 or inversin.

[0216] Antibody binding is detected by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

[0217] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many methods are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[0218] In some embodiments, an automated detection assay is utilized. Methods for the automation of immunoassays include those described in U.S. Patents 5,885,530, 4,981,785, 6,159,750, and 5,358,691, each of which is herein incorporated by reference. In some embodiments, the analysis and presentation of results is also automated. For example, in some embodiments, software that generates a prognosis based on the result of the immunoassay is utilized.

[0219] In other embodiments, the immunoassay described in U.S. Patents 5,599,677 and 5,672,480; each of which is herein incorporated by reference.

8. Kits for Analyzing Risk of NPHP Type 4 or Type 2 Disease

[0220] The present invention also provides kits for determining whether an individual contains a wild-type or variant (*e.g.*, mutant or polymorphic) allele of NPHP4, inversin, or NPHP3. In some embodiments, the kits are useful for determining whether the subject is at risk of developing NPHP type 4, type 3 or type 2 disease. The diagnostic kits are produced in a variety of ways. In some embodiments, the kits contain at least one reagent for specifically detecting a mutant NPHP4 allele or protein. In other embodiments, the kits contain at least one reagent for specifically detecting a mutant inversin allele or protein. In still other embodiments, the kits contain at least one reagent for specifically detecting a mutant NPHP3 allele or protein. In preferred embodiments, the kits contain reagents for detecting a truncation in the NPHP4, inversin or NPHP3 gene. In preferred embodiments, the reagent is a nucleic acid that hybridizes to nucleic acids containing the mutation and that does not bind to nucleic acids that do not contain the mutation. In other preferred embodiments, the reagents are primers for amplifying the region of DNA containing the mutation. In still other embodiments, the reagents are antibodies that preferentially bind either the wild-type or truncated NPHP4, inversin or NPHP3 proteins.

[0221] In some embodiments, the kit contains instructions for determining whether the subject is at risk for developing NPHP type 4, type 3 or type 2 disease. In preferred embodiments, the instructions specify that risk for developing NPHP type 4, type 3 or type 2 disease is determined by detecting the presence or absence of a mutant NPHP4, NPHP3 or inversin allele in the subject, wherein subjects having an mutant (*e.g.*, truncated) allele are at greater risk for NPHP disease.

[0222] The presence or absence of a disease-associated mutation in a NPHP4, NPHP3 or inversin gene can be used to make therapeutic or other medical decisions. For example, couples with a family history of NPHP may choose to conceive a child via *in vitro* fertilization and pre-implantation genetic screening. In this case, fertilized embryos are screened for mutant (*e.g.*, disease associated) alleles of the NPHP4, NPHP3 or inversin gene and only embryos with wild type alleles are implanted in the uterus.

[0223] In other embodiments, in utero screening is performed on a developing fetus (*e.g.*, amniocentesis or chorionic villi screening). In still other embodiments, genetic screening of newborn babies or very young children is performed. The early detection of a NPHP4, NPHP3or inversin allele known to be associated with kidney disease allows for early intervention (*e.g.*, genetic or pharmaceutical therapies).

[0224] In some embodiments, the kits include ancillary reagents such as buffering agents, nucleic acid stabilizing reagents, protein stabilizing reagents, and signal producing systems (*e.g.*, florescence generating systems as Fret systems). The test kit may be packages in any suitable manner, typically with the elements in a single container or various containers as necessary along with a sheet of instructions for carrying out the test. In some embodiments, the kits also preferably include a positive control sample.

9. Bioinformatics

[0225] In some embodiments, the present invention provides methods of determining an individual's risk of developing NPHP disease based on the presence of one or more variant alleles of NPHP4, NPHP3 or inversin. In some embodiments, the analysis of variant data is processed by a computer using information stored on a computer (*e.g.*, in a database). For example, in some embodiments, the present invention provides a bioinformatics research system comprising a plurality of computers running a multi-platform object oriented programming language (*See e.g.*, U.S. Patent 6,125,383; herein incorporated by reference). In some embodiments, one of the computers stores genetics data (*e.g.*, the risk of contacting NPHP type 4, type3 or type 2 disease associated with a given polymorphism, as well as the sequences). In some embodiments, one of the computers stores application programs (*e.g.*, for analyzing the results of detection assays). Results are then delivered to the user (*e.g.*, via one of the computers or via the internet.

[0226] For example, in some embodiments, a computer-based analysis program is used to translate the raw data generated by the detection assay (*e.g.*, the presence, absence, or amount of a given NPHP4 allele or polypeptide) into data of predictive value for a clinician. The clinician can access the predictive data using any suitable means. Thus, in some preferred embodiments, the present invention provides the further benefit that the clinician, who is not likely to be trained in genetics or molecular biology, need not understand the raw data. The data is presented directly to the clinician in its most useful form. The clinician is then able to immediately utilize the information in order to optimize the care of the subject.

[0227] The present invention contemplates any method capable of receiving, processing, and transmitting the information to and from laboratories conducting the assays, information provides, medical personal, and subjects. For example, in some embodiments of the present invention, a sample (*e.g.*, a biopsy or a serum or urine sample) is obtained from a subject and submitted to a profiling service (*e.g.*, clinical lab at a medical facility, genomic profiling business, etc.), located in any part of the world (*e.g.*, in a country different than the country where the subject resides or where the information is ultimately used) to generate raw data. Where the sample comprises a tissue or other biological sample, the subject may visit a medical center to have the sample obtained and sent to the profiling center, or subjects may collect the sample themselves (*e.g.*, a urine sample) and directly send it to a profiling center. Where the sample comprises previously determined biological information, the information may be directly sent to the profiling service by the subject (*e.g.*, an information card containing the information may be scanned by a computer and the data transmitted to a computer of the profiling center using an electronic communication systems). Once received by the profiling service, the sample is processed and a profile is produced (*i.e.*, presence of wild type or mutant NPHP4, NPHP3 or inversin genes or polypeptides), specific for the diagnostic or prognostic information desired for the subject.

[0228] The profile data is then prepared in a format suitable for interpretation by a treating clinician. For example, rather than providing raw data, the prepared format may represent a diagnosis or risk assessment (*e.g.*, likelihood of developing NPHP or a diagnosis of NPHP) for the subject, along with recommendations for particular treatment options. The data may be displayed to the clinician by any suitable method. For example, in some embodiments, the profiling service generates a report that can be printed for the clinician (*e.g.*, at the point of care) or displayed to the clinician on a computer monitor.

[0229] In some embodiments, the information is first analyzed at the point of care or at a regional facility. The raw data is then sent to a central processing facility for further analysis and/or to convert the raw data to information useful for a clinician or patient. The central processing facility provides the advantage of privacy (all data is stored in a central facility with uniform security protocols), speed, and uniformity of data analysis. The central processing facility can then control the fate of the data following treatment of the subject. For example, using an electronic communication system, the central facility can provide data to the clinician, the subject, or researchers.

[0230] In some embodiments, the subject is able to directly access the data using the electronic communication system. The subject may chose further intervention or counseling based on the results. In some embodiments, the data is used for research use. For example, the data may be used to further optimize the inclusion or elimination of markers as useful indicators of a particular condition or stage of disease.

IV. Generation of NPHP4 and Inversin Antibodies

[0231] The present invention provides isolated antibodies or antibody fragments (*e.g.*, FAB fragments). Antibodies can be generated to allow for the detection of NPHP4 protein. The antibodies may be prepared using various immunogens. In one embodiment, the immunogen is a human NPHP4 peptide to generate antibodies that recognize human NPHP4. Such antibodies include, but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, Fab expression libraries, or recombinant (*e.g.*, chimeric, humanized, etc.) antibodies, as long as it can recognize the protein. Antibodies can be produced by using a protein of the present invention as the antigen according to a conventional antibody or antiserum preparation process.

[0232] Various procedures known in the art may be used for the production of polyclonal antibodies directed against NPHP4. For the production of antibody, various host animals can be immunized by injection with the peptide corresponding to the NPHP4 epitope including but not limited to rabbits, mice, rats, sheep, goats, etc. In a preferred embodiment, the peptide is conjugated to an immunogenic carrier (*e.g.*, diphtheria toxoid, bovine serum albumin (BSA), or keyhole limpet hemocyanin (KLH)). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*).

[0233] For preparation of monoclonal antibodies directed toward NPHP4, it is contemplated that any technique that provides for the production of antibody molecules by continuous cell lines in culture will find use with the present invention (*See e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press*, Cold Spring Harbor, NY). These include but are not limited to the hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, Nature 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (*See e.g.*, Kozbor *et al.*, Immunol. Tod., 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al., in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]).

[0234] In an additional embodiment of the invention, monoclonal antibodies are produced in germ-free animals utilizing technology such as that described in PCT/US90/02545). Furthermore, it is contemplated that human antibodies will be generated by human hybridomas (Cote *et al.*, Proc. Natl. Acad. Sci. USA 80:2026-2030 [1983]) or by transforming human B cells with EBV virus *in vitro* (Cole *et al.*, *in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96 [1985]).

[0235] In addition, it is contemplated that techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; herein incorporated by reference) will find use in producing NPHP4 specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse *et al.*, Science 246:1275-1281 [1989]) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for NPHP4.

[0236] In other embodiments, the present invention contemplated recombinant antibodies or fragments thereof to the proteins of the present invention. Recombinant antibodies include, but are not limited to, humanized and chimeric antibodies. Methods for generating recombinant antibodies are known in the art (*See e.g.*, U.S. Patents 6,180,370 and 6,277,969 and "Monoclonal Antibodies" H. Zola, BIOS Scientific Publishers Limited 2000. Springer-Verlay New York, Inc., New York; each of which is herein incorporated by reference).

[0237] It is contemplated that any technique suitable for producing antibody fragments will find use in generating antibody fragments that contain the idiotype (antigen binding region) of the antibody molecule. For example, such fragments include but are not limited to: F(ab')2 fragment that can be produced by pepsin digestion of the antibody molecule; Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and Fab fragments that can be generated by treating the antibody molecule with papain and a reducing agent.

[0238] In the production of antibodies, it is contemplated that screening for the desired antibody will be accomplished by techniques known in the art (*e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, *in situ* immunoassays (*e.g.*, using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

[0239] In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. As is well known in the art, the immunogenic peptide should be provided free of the carrier molecule used in any immunization protocol. For example, if the peptide was conjugated to KLH, it may be conjugated to BSA, or used directly, in a screening assay.)

[0240] Additionally, using the above methods, antibodies can be generated that recognize the variant forms of NPHP4 or inversin, while not recognizing the wild type forms of the NPHP4 or inversin proteins.

[0241] The foregoing antibodies can be used in methods known in the art relating to the localization and structure of NPHP4 and inversin (*e.g.*, for Western blotting, immunoprecipitaion and immunocytochemistry, see Examples 3-6), measuring levels thereof in appropriate biological samples, etc. The antibodies can be used to detect NPHP4 or inversin in a biological sample from an individual. The biological sample can be a biological fluid, such as, but not limited to, blood, serum, plasma, interstitial fluid, urine, cerebrospinal fluid, and the like, containing cells.

[0242] The biological samples can then be tested directly for the presence of human NPHP4 using an appropriate strategy (*e.g.*, ELISA or radioimmunoassay) and format (*e.g.*, microwells, dipstick (*e.g.*, as described in International Patent Publication WO 93/03367), etc. Alternatively, proteins in the sample can be size separated (*e.g.*, by polyacrylamide gel electrophoresis (PAGE), in the presence or not of sodium dodecyl sulfate (SDS), and the presence of NPHP4 detected by immunoblotting (Western blotting). Immunoblotting techniques are generally more effective with antibodies generated against a peptide corresponding to an epitope of a protein, and hence, are particularly suited to the present invention.

[0243] Another method uses antibodies as agents to alter signal transduction. Specific antibodies that bind to the binding domains of NPHP4 or inversin or other proteins involved in intracellular signaling can be used to inhibit the interaction between the various proteins and their interaction with other ligands. Antibodies that bind to the complex can also be used therapeutically to inhibit interactions of the protein complex in the signal transduction pathways leading to the various physiological and cellular effects of NPHP. Such antibodies can also be used diagnostically to measure abnormal expression of NPHP4 or inversin, or the aberrant formation of protein complexes, which may be indicative of a disease state.

V. Gene Therapy Using NPHP4 and Inversin

[0244] The present invention also provides methods and compositions suitable for gene therapy to alter NPHP4 or inversin expression, production, or function. As described above, the present invention provides human NPHP4 genes and provides methods of obtaining NPHP4 genes from other species. Thus, the methods described below are generally applicable across many species. In some embodiments, it is contemplated that the gene therapy is performed by providing a subject with a wild-type allele of NPHP4 or inversin (*i.e.*, an allele that does not contain a NPHP disease causing polymorphisms or mutations, See Example 6). Subjects in need of such therapy are identified by the methods described above.

[0245] Viral vectors commonly used for *in vivo* or *ex vivo* targeting and therapy procedures are DNA-based vectors and retroviral vectors. Methods for constructing and using viral vectors are known in the art (*See e.g.*, Miller and Rosman, BioTech., 7:980-990 [1992]). Preferably, the viral vectors are replication defective, that is, they are unable to replicate autonomously in the target cell. In general, the genome of the replication defective viral vectors that are used within the scope of the present invention lack at least one region that is necessary for the replication of the virus in the infected cell. These regions can either be eliminated (in whole or in part), or be rendered non-functional by any technique known to a person skilled in the art. These techniques include the total removal, substitution (by other sequences, in particular by the inserted nucleic acid), partial deletion or addition of one or more bases to an essential (for replication) region. Such techniques may be performed *in vitro* (*i.e.*, on the isolated DNA) or *in situ*, using the techniques of genetic manipulation or by treatment with mutagenic agents.

[0246] Preferably, the replication defective virus retains the sequences of its genome that are necessary for encapsidating the viral particles. DNA viral vectors include an attenuated or defective DNA viruses, including, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, that entirely or almost entirely lack viral genes, are preferred, as defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, a specific tissue can be specifically targeted. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector (Kaplitt *et al.*, Mol. Cell. Neurosci., 2:320-330 [1991]), defective herpes virus vector lacking a glycoprotein L gene (*See e.g.*, Patent Publication RD 371005 A), or other defective herpes virus vectors (*See e.g.*, WO 94/21807; and WO 92/05263); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet *et al.* (J. Clin. Invest., 90:626-630 [1992]; *See also*, La Salle *et al.*, Science 259:988-990 [1993]); and a defective adeno-associated virus vector (Samulski *et al.*, J. Virol., 61:3096-3101 [1987]; Samulski *et al.*, J. Virol., 63:3822-3828 [1989]; and Lebkowski *et al.*, Mol. Cell. Biol., 8:3988-3996 [1988]).

[0247] Preferably, for *in vivo* administration, an appropriate immunosuppressive treatment is employed in conjunction with the viral vector (*e.g.*, adenovirus vector), to avoid immuno-deactivation of the viral vector and transfected cells. For example, immunosuppressive cytokines, such as interleukin-12 (IL-12), interferon-gamma (IFN-γ), or anti-CD4 antibody, can be administered to block humoral or cellular immune responses to the viral vectors. In addition, it is advantageous to employ a viral vector that is engineered to express a minimal number of antigens.

[0248] In a preferred embodiment, the vector is an adenovirus vector. Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a nucleic acid of the invention to a variety of cell types. Various serotypes of adenovirus exist. Of these serotypes, preference is given, within the scope of the present invention, to type 2 or type 5 human adenoviruses (Ad 2 or Ad 5), or adenoviruses of animal origin (*See e.g.*, WO 94/26914). Those adenoviruses of animal origin that can be used within the scope of the present invention include adenoviruses of canine, bovine, murine (*e.g.*, Mav1, Beard *et al.*, Virol., 75-81 [1990]), ovine, porcine, avian, and simian (*e.g.*, SAV) origin. Preferably, the adenovirus of animal origin is a canine adenovirus, more preferably a CAV2 adenovirus (*e.g.* Manhattan or A26/61 strain (ATCC VR-800)).

[0249] Preferably, the replication defective adenoviral vectors of the invention comprise the ITRs, an encapsidation sequence and the nucleic acid of interest. Still more preferably, at least the E1 region of the adenoviral vector is non-functional. The deletion in the E1 region preferably extends from nucleotides 455 to 3329 in the sequence of the Ad5 adenovirus (*Pvu*II-*Bgl*II fragment) or 382 to 3446 (*Hinf*II-*Sau*3A fragment). Other regions may also be modified, in particular the E3 region (*e.g.*, WO 95/02697), the E2 region (*e.g.*, WO 94/28938), the E4 region (*e.g.*, WO 94/28152, WO 94/12649 and WO 95/02697), or in any of the late genes L1-L5.

[0250] In a preferred embodiment, the adenoviral vector has a deletion in the E1 region (Ad 1.0). Examples of E1-deleted adenoviruses are disclosed in EP 185,573, the contents of which are incorporated herein by reference. In another preferred embodiment, the adenoviral vector has a deletion in the E1 and E4 regions (Ad 3.0). Examples of E1/E4-deleted adenoviruses are disclosed in WO 95/02697 and WO 96/22378. In still another preferred embodiment, the adenoviral vector has a deletion in the E1 region into which the E4 region and the nucleic acid sequence are inserted.

[0251] The replication defective recombinant adenoviruses according to the invention can be prepared by any technique known to the person skilled in the art (*See e.g.*, Levrero *et al.*, Gene 101:195 [1991]; EP 185 573; and Graham, EMBO J., 3:2917 [1984]). In particular, they can be prepared by homologous recombination between an adenovirus and a plasmid that carries, *inter alia*, the DNA sequence of interest. The homologous recombination is accomplished following co-transfection of the adenovirus and plasmid into an appropriate cell line. The cell line that is employed should preferably (i) be transformable by the elements to be used, and (ii) contain the sequences that are able to complement the part of the genome of the replication defective adenovirus, preferably in integrated form in order to avoid the risks of recombination. Examples of cell lines that may be used are the human embryonic kidney cell line 293 (Graham *et al.*, J. Gen. Virol., 36:59 [1977]), which contains the left-hand portion of the genome of an Ad5 adenovirus (12%) integrated into its genome, and cell lines that are able to complement the E1 and E4 functions, as described in applications WO 94/26914 and WO 95/02697. Recombinant adenoviruses are recovered and purified using standard molecular biological techniques that are well known to one of ordinary skill in the art.

[0252] The adeno-associated viruses (AAV) are DNA viruses of relatively small size that can integrate, in a stable and site-specific manner, into the genome of the cells that they infect. They are able to infect a wide spectrum of cells without inducing any effects on cellular growth, morphology or differentiation, and they do not appear to be involved in human pathologies. The AAV genome has been cloned, sequenced and characterized. It encompasses approximately 4700 bases and contains an inverted terminal repeat (ITR) region of approximately 145 bases at each end, which serves as an origin of replication for the virus. The remainder of the genome is divided into two essential regions that carry the encapsidation functions: the left-hand part of the genome, that contains the *rep* gene involved in viral replication and expression of the viral genes; and the right-hand part of the genome, that contains the *cap* gene encoding the capsid proteins of the virus.

[0253] The use of vectors derived from the AAVs for transferring genes *in vitro* and *in vivo* has been described (*See e.g.,* WO 91/18088; WO 93/09239; US Pat. No. 4,797,368; US Pat. No., 5,139,941; and EP 488 528, all of which are herein incorporated by reference). These publications describe various AAV-derived constructs in which the *rep* and/or *cap* genes are deleted and replaced by a gene of interest, and the use of these constructs for transferring the gene of interest *in vitro* (into cultured cells) or *in vivo* (directly into an organism). The replication defective recombinant AAVs according to the invention can be prepared by co-transfecting a plasmid containing the nucleic acid sequence of interest flanked by two AAV inverted terminal repeat (ITR) regions, and a plasmid carrying the AAV encapsidation genes (*rep* and *cap* genes), into a cell line that is infected with a human helper virus (for example an adenovirus). The AAV recombinants that are produced are then purified by standard techniques.

[0254] In another embodiment, the gene can be introduced in a retroviral vector (*e.g.*, as described in U.S. Pat. Nos. 5,399,346, 4,650,764, 4,980,289 and 5,124,263; all of which are herein incorporated by reference; Mann *et al.*, Cell 33:153 [1983]; Markowitz *et al.*, J. Virol., 62:1120 [1988]; PCT/US95/14575; EP 453242; EP178220; Bernstein *et al.* Genet. Eng., 7:235 [1985]; McCormick, BioTechnol., 3:689 [1985]; WO 95/07358; and Kuo *et al.*, Blood 82:845 [1993]). The retroviruses are integrating viruses that infect dividing cells. The retrovirus genome includes two LTRs, an encapsidation sequence and three coding regions (*gag, pol* and *env*). In recombinant retroviral vectors, the *gag, pol* and *env* genes are generally deleted, in whole or in part, and replaced with a heterologous nucleic acid sequence of interest. These vectors can be constructed from different types of retrovirus, such as, HIV, MoMuLV ("murine Moloney leukemia virus" MSV ("murine Moloney sarcoma virus"), HaSV ("Harvey sarcoma virus"); SNV ("spleen necrosis virus"); RSV ("Rous sarcoma virus") and Friend virus. Defective retroviral vectors are also disclosed in WO 95/02697.

[0255] In general, in order to construct recombinant retroviruses containing a nucleic acid sequence, a plasmid is constructed that contains the LTRs, the encapsidation sequence and the coding sequence. This construct is used to transfect a packaging cell line, which cell line is able to supply in trans the retroviral functions that are deficient in the plasmid. In general, the packaging cell lines are thus able to express the *gag, pol* and *env* genes. Such packaging cell lines have been described in the prior art, in particular the cell line PA317 (US Pat. No. 4,861,719, herein incorporated by reference), the PsiCRIP cell line (*See,* WO90/02806), and the GP+envAm-12 cell line (*See,* WO89/07150). In addition, the recombinant retroviral vectors can contain modifications within the LTRs for suppressing transcriptional activity as well as extensive encapsidation sequences that may include a part of the *gag* gene (Bender *et al.*, J. Virol., 61:1639 [1987]). Recombinant retroviral vectors are purified by standard techniques known to those having ordinary skill in the art.

[0256] Alternatively, the vector can be introduced *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner *et. al.*, Proc. Natl. Acad. Sci. USA 84:7413-7417 [1987]; *See also,* Mackey, *et al.*, Proc. Natl. Acad. Sci. USA 85:8027-8031 [1988]; Ulmer *et al.*, Science 259:1745-1748 [1993]). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, Science 337:387-388 [1989]). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in WO95/18863 and WO96/17823, and in U.S. Pat. No. 5,459,127, herein incorporated by reference.

[0257] Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (*e.g.*, WO95/21931), peptides derived from DNA binding proteins (*e.g.*, WO96/25508), or a cationic polymer (*e.g.*, WO95/21931).

[0258] It is also possible to introduce the vector *in vivo* as a naked DNA plasmid. Methods for formulating and administering naked DNA to mammalian muscle tissue are disclosed in U.S. Pat. Nos. 5,580,859 and 5,589,466, both of which are herein incorporated by reference.

[0259] DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, including but not limited to transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (*See e.g.*, Wu *et al.*, J. Biol. Chem., 267:963 [1992]; Wu and Wu, J. Biol. Chem., 263:14621 [1988]; and Williams *et al.*, Proc. Natl. Acad. Sci. USA 88:2726

[1991]). Receptor-mediated DNA delivery approaches can also be used (Curiel *et al.*, Hum. Gene Ther., 3:147 [1992]; and Wu and Wu, J. Biol. Chem., 262:4429 [1987]).

VI. Transgenic Animals Expressing Exogenous NPHP4 Genes and Homologs, Mutants, and Variants Thereof

[0260] The present invention contemplates the generation of transgenic animals comprising an exogenous NPHP4 gene or inversin gene or homologs, mutants, or variants thereof. In preferred embodiments, the transgenic animal displays an altered phenotype as compared to wild-type animals. In some embodiments, the altered phenotype is the overexpression of mRNA for a NPHP4 gene or inversin gene as compared to wild-type levels of NPHP4 or inversin expression. In other embodiments, the altered phenotype is the decreased expression of mRNA for an endogenous NPHP4 gene or inversin gene as compared to wild-type levels of endogenous NPHP4 or inversin expression. In some preferred embodiments, the transgenic animals comprise mutant (*e.g.*, truncated) alleles of NPHP4 or inversin. Methods for analyzing the presence or absence of such phenotypes include Northern blotting, mRNA protection assays, and RT-PCR. In other embodiments, the transgenic mice have a knock out mutation of the NPHP4 gene or inversin gene. In preferred embodiments, the transgenic animals display a NPHP disease phenotype.

[0261] Such animals find use in research applications (*e.g.*, identifying signaling pathways involved in NPHP), as well as drug screening applications (*e.g.*, to screen for drugs that prevents NPHP disease. For example, in some embodiments, test compounds (*e.g.*, a drug that is suspected of being useful to treat NPHP disease) and control compounds (*e.g.*, a placebo) are administered to the transgenic animals and the control animals and the effects evaluated. The effects of the test and control compounds on disease symptoms are then assessed.

[0262] The transgenic animals can be generated via a variety of methods. In some embodiments, embryonal cells at various developmental stages are used to introduce transgenes for the production of transgenic animals. Different methods are used depending on the stage of development of the embryonal cell. The zygote is the best target for micro-injection. In the mouse, the male pronucleus reaches the size of approximately 20 micrometers in diameter, which allows reproducible injection of 1-2 picoliters (pl) of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host genome before the first cleavage (Brinster *et al.*, Proc. Natl. Acad. Sci. USA 82:4438-4442 [1985]). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. U.S. Patent No. 4,873,191 describes a method for the micro-injection of zygotes; the disclosure of this patent is incorporated herein in its entirety.

[0263] In other embodiments, retroviral infection is used to introduce transgenes into a non-human animal. In some embodiments, the retroviral vector is utilized to transfect oocytes by injecting the retroviral vector into the perivitelline space of the oocyte (U.S. Pat. No. 6,080,912, incorporated herein by reference). In other embodiments, the developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Janenich, Proc. Natl. Acad. Sci. USA 73:1260 [1976]). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan *et al.*, in *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [1986]). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner *et al.*, Proc. Natl. Acad Sci. USA 82:6927 [1985]). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart, *et al.*, EMBO J., 6:383 [1987]). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner *et al.*, Nature 298:623 [1982]). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of cells that form the transgenic animal. Further, the founder may contain various retroviral insertions of the transgene at different positions in the genome that generally will segregate in the offspring. In addition, it is also possible to introduce transgenes into the germline, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner *et al.*, *supra* [1982]). Additional means of using retroviruses or retroviral vectors to create transgenic animals known to the art involves the micro-injection of retroviral particles or mitomycin C-treated cells producing retrovirus into the perivitelline space of fertilized eggs or early embryos (PCT International Application WO 90/08832 [1990], and Haskell and Bowen, Mol. Reprod. Dev., 40:386 [1995]).

[0264] In other embodiments, the transgene is introduced into embryonic stem cells and the transfected stem cells are utilized to form an embryo. ES cells are obtained by culturing pre-implantation embryos *in vitro* under appropriate conditions (Evans *et al.*, Nature 292:154 [1981]; Bradley *et al.*, Nature 309:255 [1984]; Gossler *et al.*, Proc. Acad. Sci. USA 83:9065 [1986]; and Robertson *et al.*, Nature 322:445 [1986]). Transgenes can be efficiently introduced into the ES cells by DNA transfection by a variety of methods known to the art including calcium phosphate co-precipitation, protoplast or spheroplast fusion, lipofection and DEAE-dextran-mediated transfection. Transgenes may also be introduced into ES cells by retrovirus-mediated transduction or by micro-injection. Such transfected ES cells can thereafter colonize an embryo following their introduction into the blastocoel of a blastocyst-stage embryo and contribute to the germ line of the resulting chimeric animal (for review, *See*, Jaenisch, Science 240:1468 [1988]). Prior to the introduction of transfected ES cells into the blastocoel, the transfected ES cells may be subjected to various selection protocols to enrich for ES cells which have integrated the transgene assuming that the transgene provides a means for such selection. Alternatively, the polymerase chain reaction may be used to screen for ES cells that have integrated the transgene. This technique obviates the need for growth of the transfected ES cells under appropriate selective conditions prior to transfer into the blastocoel.

[0265] In still other embodiments, homologous recombination is utilized to knock-out gene function or create deletion mutants (*e.g.*, mutants in which the LRRs of NPHP4 are deleted). Methods for homologous recombination are described in U.S. Pat. No. 5,614,396, incorporated herein by reference.

VIII. Drug Screening Using NPHP4 and Inversin

[0266] As described herein, it is contemplated that nephroretinin, inversin and nephrocystin interact within a novel shared pathogenic pathway (e.g., as shown in Examples 3-5). Accordingly, in some embodiments, the isolated nucleic acid sequences of NPHP4 (*e.g.*, SEQ ID NOS: 1, 5, 7, 9, 11, 13, 15, 17, and 19) and inversin (*e.g.*, SEQ ID Nos: 24, 26, 28, 30, 34, 36, 38 and 40) are used in drug screening applications for compounds that alter (*e.g.*, enhance) signaling within the pathway.

A. Identification of Binding Partners

[0267] In some embodiments, binding partners of NPHP4 amino acids and inversin amino acids are identified. In some embodiments, the NPHP4 nucleic acid sequence (*e.g.*, SEQ ID NOS: 1, 5, 7, 9, 11, 13, 15, 17, and 19) and inversin nucleic acid sequences (*e.g.*, SEQ ID Nos: 21, 23, 25, 27, 29, 33, 35, 37 and 39) or fragments thereof are used in yeast two-hybrid screening assays. For example, in some embodiments, the nucleic acid sequences are subcloned into *pGPT9* (Clontech, La Jolla, CA) to be used as a bait in a yeast-2-hybrid screen for protein-protein interaction of a human fetal kidney cDNA library (Fields and Song *Nature* 340:245 -246, 1989; herein incorporated by reference). In other embodiments, phage display is used to identify binding partners (Parmley and Smith *Gene* 73 : 305-318, [1988]; herein incorporated by reference).

B. Drug Screening

[0268] The present invention provides methods and compositions for using NPHP4 and inversin as a target for screening drugs that can alter, for example, interaction between NPHP4 and inversin and their binding partners (*e.g.*, those identified using the above methods)

[0269] In one screening method, the two-hybrid system is used to screen for compounds (*e.g.*, drug) capable of altering (*e.g.*, inhibiting) NPHP4 function(s) or inversin function(s) (*e.g.*, interaction with a binding partner) *in vitro* or *in vivo*. In one embodiment, a GAL4 binding site, linked to a reporter gene such as *lac*Z, is contacted in the presence and absence of a candidate compound with a GAL4 binding domain linked to a NPHP4 fragment or a inversin fragment and a GAL4 transactivation domain II linked to a binding partner fragment. Expression of the reporter gene is monitored and a decrease in the expression is an indication that the candidate compound inhibits the interaction of NPHP4 or inversin with the binding partner. Alternately, the effect of candidate compounds on the interaction of NPHP4 with other proteins (*e.g.*, proteins known to interact directly or indirectly with the binding partner) can be tested in a similar manner.

[0270] In another screening method, candidate compounds are evaluated for their ability to alter NPHP4 signaling or inversin signaling by contacting NPHP4 or inversin, binding partners, binding partner-associated proteins, or fragments thereof, with the candidate compound and determining binding of the candidate compound to the peptide. The protein or protein fragments is/are immobilized using methods known in the art such as binding a GST-NPHP4 or a GST-inversin fusion protein to a polymeric bead containing glutathione. A chimeric gene encoding a GST fusion protein is constructed by fusing DNA encoding the polypeptide or polypeptide fragment of interest to the DNA encoding the carboxyl terminus of GST (*See e.g.*, Smith *et al.*, Gene 67:31 [1988]). The fusion construct is then transformed into a suitable expression system (*e.g.*, *E. coli* XA90) in which the expression of the GST fusion protein can be induced with isopropyl-β-D-thiogalactopyranoside (IPTG). Induction with IPTG should yield the fusion protein as a major constituent of soluble, cellular proteins. The fusion proteins can be purified by methods known to those skilled in the art, including purification by glutathione affinity chromatography. Binding of the candidate compound to the proteins or protein fragments is correlated with the ability of the compound to disrupt the signal transduction pathway and thus regulate NPHP4 or inversin physiological effects (*e.g.*, kidney disease).

[0271] In another screening method, one of the components of the NPHP4or inversin/binding partner signaling system, is immobilized. Polypeptides can be immobilized using methods known in the art, such as adsorption onto a plastic microtiter plate or specific binding of a GST-fusion protein to a polymeric bead containing glutathione. For example, GST-NPHP4 or GST-inversin is bound to glutathione-Sepharose beads. The immobilized peptide is then contacted with another peptide with which it is capable of binding in the presence and absence of a candidate compound. Unbound peptide is then removed and the complex solubilized and analyzed to determine the amount of bound labeled peptide. A decrease in binding is an indication that the candidate compound inhibits the interaction of NPHP4 or inversin with the other peptide. A variation of this method allows for the screening of compounds that are capable of disrupting a previously-formed protein/protein complex. For example, in some embodiments a complex comprising NPHP4 or inversin or fragments thereof bound to another peptide is immobilized as described above and contacted with a candidate compound. The dissolution of the complex by the candidate compound correlates with the ability of the compound to disrupt or inhibit the interaction between NPHP4 or inversin and the other peptide.

[0272] Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to NPHP4 peptides or inversin peptides and is described in detail in WO 84/03564, incorporated herein by reference. Briefly, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are then reacted with NPHP4 peptides or inversin peptides and washed. Bound NPHP4 peptides or inversin peptides are then detected by methods well known in the art.

[0273] Another technique uses NPHP4 antibodies or inversin antibodies, generated as discussed above. Such antibodies capable of specifically binding to NPHP4 peptides or inversin peptides compete with a test compound for binding to NPHP4 or inversin. In this manner, the antibodies can be used to detect the presence of any peptide that shares one or more antigenic determinants of the NPHP4 peptide or inversin peptide.

[0274] The present invention contemplates many other means of screening compounds. The examples provided above are presented merely to illustrate a range of techniques available. One of ordinary skill in the art will appreciate that many other screening methods can be used.

[0275] In particular, the present invention contemplates the use of cell lines transfected with NPHP4 and inversin and variants thereof for screening compounds for activity, and in particular to high throughput screening of compounds from combinatorial libraries (*e.g.*, libraries containing greater than 10^4 compounds). The cell lines of the present invention can be used in a variety of screening methods. In some embodiments, the cells can be used in second messenger assays that monitor signal transduction following activation of cell-surface receptors. In other embodiments, the cells can be used in reporter gene assays that monitor cellular responses at the transcription/translation level. In still further embodiments, the cells can be used in cell proliferation assays to monitor the overall growth/no growth response of cells to external stimuli.

[0276] In second messenger assays, the host cells are preferably transfected as described above with vectors encoding NPHP4 or inversin or variants or mutants thereof. The host cells are then treated with a compound or plurality of compounds (*e.g.*, from a combinatorial library) and assayed for the presence or absence of a response. It is contemplated that at least some of the compounds in the combinatorial library can serve as agonists, antagonists, activators, or inhibitors of the protein or proteins encoded by the vectors. It is also contemplated that at least some of the compounds in the combinatorial library can serve as agonists, antagonists, activators, or inhibitors of protein acting upstream or downstream of the protein encoded by the vector in a signal transduction pathway.

[0277] In some embodiments, the second messenger assays measure fluorescent signals from reporter molecules that respond to intracellular changes (*e.g.*, Ca^{2+} concentration, membrane potential, pH, IP_3, cAMP, arachidonic acid release) due to stimulation of membrane receptors and ion channels (*e.g.*, ligand gated ion channels; *see* Denyer *et al.*, Drug Discov. Today 3:323 [1998]; and Gonzales *et al.*, Drug. Discov. Today 4:431-39 [1999]). Examples of reporter molecules include, but are not limited to, FRET (florescence resonance energy transfer) systems (*e.g.*, Cuo-lipids and oxonols, EDAN/DABCYL), calcium sensitive indicators (*e.g.*, Fluo-3, FURA 2, INDO 1, and FLUO3/AM, BAPTA AM), chloride-sensitive indicators (*e.g.*, SPQ, SPA), potassium-sensitive indicators (*e.g.*, PBFI), sodium-sensitive indicators (*e.g.*, SBFI), and pH sensitive indicators (*e.g.*, BCECF).

[0278] In general, the host cells are loaded with the indicator prior to exposure to the compound. Responses of the host cells to treatment with the compounds can be detected by methods known in the art, including, but not limited to, fluorescence microscopy, confocal microscopy (*e.g.*, FCS systems), flow cytometry, microfluidic devices, FLIPR systems (*See, e.g.*, Schroeder and Neagle,

J. Biomol. Screening 1:75 [1996]), and plate-reading systems. In some preferred embodiments, the response (*e.g.*, increase in fluorescent intensity) caused by compound of unknown activity is compared to the response generated by a known agonist and expressed as a percentage of the maximal response of the known agonist. The maximum response caused by a known agonist is defined as a 100% response. Likewise, the maximal response recorded after addition of an agonist to a sample containing a known or test antagonist is detectably lower than the 100% response.

[0279] The cells are also useful in reporter gene assays. Reporter gene assays involve the use of host cells transfected with vectors encoding a nucleic acid comprising transcriptional control elements of a target gene (*i.e.*, a gene that controls the biological expression and function of a disease target) spliced to a coding sequence for a reporter gene. Therefore, activation of the target gene results in activation of the reporter gene product. In some embodiments, the reporter gene construct comprises the 5' regulatory region (*e.g.*, promoters and/or enhancers) of a protein whose expression is controlled by NPHP4 or inversin in operable association with a reporter gene (See Example 4 and Inohara *et al.*, J. Biol. Chem. 275:27823 [2000] for a description of the luciferase reporter construct pBVIx-Luc). Examples of reporter genes finding use in the present invention include, but are not limited to, chloramphenicol transferase, alkaline phosphatase, firefly and bacterial luciferases, β-galactosidase, β-lactamase, and green fluorescent protein. The production of these proteins, with the exception of green fluorescent protein, is detected through the use of chemiluminescent, colorimetric, or bioluminecent products of specific substrates (*e.g.*, X-gal and luciferin). Comparisons between compounds of known and unknown activities may be conducted as described above.

[0280] Specifically, the present invention provides screening methods for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to NPHP4 or inversin of the present invention, have an inhibitory (or stimulatory) effect on, for example, NPHP4 or inversin expression or NPHP4 or inversin activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a NPHP4 or inversin substrate. Compounds thus identified can be used to modulate the activity of target gene products (*e.g.*, NPHP4 or inversin genes) either directly or indirectly in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions. Compounds which stimulate the activity of a variant NPHP4 or variant inversin or mimic the activity of a non-functional variant are particularly useful in the treatment of cystic kidney diseases (*e.g.*, NPHP).

[0281] In one embodiment, the invention provides assays for screening candidate or test compounds that are substrates of a NPHP4 protein or inversin protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate the activity of a NPHP4 protein or inversin protein or polypeptide or a biologically active portion thereof.

[0282] The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone, which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.*, Zuckennann *et al.*, J. Med. Chem. 37: 2678 [1994]); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are preferred for use with peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) Anticancer Drug Des. 12:145).

[0283] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.*, Proc. Natl. Acad. Sci. U.S.A. 90:6909 [1993]; Erb *et al.*, Proc. Nad. Acad. Sci. USA 91:11422 [1994]; Zuckermann *et al.*, J. Med. Chem. 37:2678 [1994]; Cho *et al.*, Science 261:1303 [1993]; Carrell *et al.*, Angew. Chem. Int. Ed. Engl. 33.2059 [1994]; Carell *et al.*, Angew. Chem. Int. Ed. Engl. 33:2061 [1994]; and Gallop *et al.*, J. Med. Chem. 37:1233 [1994].

[0284] Libraries of compounds may be presented in solution (*e.g.*, Houghten, Biotechniques 13:412-421 [1992]), or on beads (Lam, Nature 354:82-84 [1991]), chips (Fodor, Nature 364:555-556 [1993]), bacteria or spores (U.S. Patent No. 5,223,409; herein incorporated by reference), plasmids (Cull *et al.*, Proc. Nad. Acad. Sci. USA 89:18651869 [1992]) or on phage (Scott and Smith, Science 249:386-390 [1990]; Devlin Science 249:404-406 [1990]; Cwirla *et al.*, Proc. Natl. Acad. Sci. 87:6378-6382 [1990]; Felici, J. Mol. Biol. 222:301 [1991]).

[0285] In one embodiment, an assay is a cell-based assay in which a cell that expresses a NPHP4 or inversin protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate NPHP4activity or inversin activity is determined. Determining the ability of the test compound to modulate NPHP4 activity or inversin activity can be accomplished by monitoring, for example, changes in enzymatic activity. The cell, for example, can be of mammalian origin.

[0286] The ability of the test compound to modulate NPHP4 binding or inversin binding to a compound, *e.g.*, a NPHP4 substrate or inversin substrate, can also be evaluated. This can be accomplished, for example, by coupling the compound, *e.g.*, the substrate, with a radioisotope or enzymatic label such that binding of the compound, *e.g.*, the substrate, to NPHP4 or inversin can be determined by detecting the labeled compound, *e.g.*, substrate, in a complex.

[0287] Alternatively, the NPHP4 or inversin is coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate NPHP4 binding or inversin binding to a NPHP4 substrate or inversin substrate in a complex. For example, compounds (*e.g.*, substrates) can be labeled with ^{125}I, ^{35}S ^{14}C or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

[0288] The ability of a compound (*e.g.*, a NPHP4 substrate or inversin substrate) to interact with NPHP4 or inversin with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with a NPHP4 or inversin without the labeling of either the compound or the NPHP4 (McConnell *et al.* Science 257:1906-1912 [1992]). As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and NPHP4 or inversin.

[0289] In yet another embodiment, a cell-free assay is provided in which a NPHP4 protein or inversin protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the NPHP4 protein or inversin protein or a biologically active portion thereof is evaluated. Preferred biologically active portions of the NPHP4 proteins or inversin proteins to be used in assays of the present invention include fragments that participate in interactions with substrates or other proteins, *e.g.*, fragments with high surface probability scores.

[0290] Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

[0291] The interaction between two molecules can also be detected, *e.g.*, using fluorescence energy transfer (FRET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos *et al.*, U.S. Patent No. 4,968,103; each of which is herein incorporated by reference). A fluorophore label is selected such that a first donor molecule's emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy.

[0292] Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in 1 5 the assay should be maximal. An FRET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

[0293] In another embodiment, determining the ability of the NPHP4 protein or inversin protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (*see*, *e.g.*, Sjolander and Urbaniczky, Anal. Chem. 63:2338-2345 [1991] and Szabo *et al.* Curr. Opin. Struct. Biol. 5:699-705 [1995]). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal that can be used as an indication of real-time reactions between biological molecules.

[0294] In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

[0295] It may be desirable to immobilize NPHP4 or inversin, an anti-NPHP4 or anti-inversin antibody or their target molecules to facilitate separation of complexed from non-complexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a NPHP4 protein or inversin protein, or interaction of a NPHP4 protein or inversin protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase-NPHP4 or glutathione-S-transferase-inversin fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione Sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione-derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or NPHP4 protein or inversin protein, and the mixture incubated under conditions conducive for complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above.

[0296] Alternatively, the complexes can be dissociated from the matrix, and the level of NPHP4 or inversin binding or activity determined using standard techniques. Other techniques for immobilizing either NPHP4 protein or inversin protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated NPHP4 or inversin protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, EL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

[0297] In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-IgG antibody).

[0298] This assay is performed utilizing antibodies reactive with NPHP4 protein or inversin protein or target molecules but which do not interfere with binding of the NPHP4 protein or inversin protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or NPHP4 protein or inversin protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the

GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the NPHP4 protein or inversin protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the NPHP4 protein or inversin protein or target molecule.

[0299] Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including, but not limited to: differential centrifugation (see, for example, Rivas and Minton, Trends Biochem Sci 18:284-7 [1993]); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (*see, e.g.*, Ausubel *et al.*, eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York.); and immunoprecipitation (*see*, for example, Ausubel *et al.*, eds. Current Protocols in Molecular Biology 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (*See e.g.*, Heegaard J. Mol. Recognit 11:141-8 [1998]; Hageand Tweed J. Chromatogr. Biomed. Sci. Appl 699:499-525 [1997]). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

[0300] The assay can include contacting the NPHP4 protein or inversin protein or biologically active portion thereof with a known compound that binds the NPHP4 or inversin to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a NPHP4 protein or inversin protein, wherein determining the ability of the test compound to interact with a NPHP4 protein or inversin protein includes determining the ability of the test compound to preferentially bind to NPHP4 or inversin or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

[0301] To the extent that NPHP4 or inversin can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins, inhibitors of such an interaction are useful. A homogeneous assay can be used to identify inhibitors.

[0302] For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared such that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496, herein incorporated by reference, that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified. Alternatively, NPHP4 protein or inversin protein can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.*, Cell 72:223-232 [1993]; Madura *et al.*, J. Biol. Chem. 268.12046-12054 [1993]; Bartel *et al.*, Biotechniques 14:920-924 [1993]; Iwabuchi *et al.*, Oncogene 8:1693-1696 [1993]; and Brent W0 94/10300; each of which is herein incorporated by reference), to identify other proteins, that bind to or interact with NPHP4 or inversin ("NPHP4-binding proteins" or "NPHP4-bp" or "inversin-binding proteins" or "inversin-bp") and are involved in NPHP4 activity or inversin activity. Such NPHP4-bps or inversin-bps can be activators or inhibitors of signals by the NPHP4 proteins or inversin proteins or targets as, for example, downstream elements of a NPHP4-mediated or inversin-mediated signaling pathway.

[0303] Modulators of NPHP4 expression or inversin expression can also be identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of NPHP4 mRNA or protein or inversin mRNA or protein evaluated relative to the level of expression of NPHP4 mRNA or protein or inversin mRNA or protein in the absence of the candidate compound. When expression of NPHP4 mRNA or protein or inversin mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of NPHP4 mRNA or protein or inversin mRNA or protein expression. Alternatively, when expression of NPHP4 mRNA or protein or inversin mRNA or protein is less (i.e., statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of NPHP4 mRNA or protein or inversin mRNA or protein expression. The level of NPHP4 mRNA or protein or inversin mRNA or protein expression can be determined by methods described herein for detecting NPHP4 mRNA or protein or inversin mRNA or protein.

[0304] A modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a NPHP4 protein or inversin protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for a disease (*e.g.*, an animal with kidney disease; See *e.g.*, Hildenbrandt and Otto, J. Am. Soc. Nephrol. 11:1753 [2000]).

C. Therapeutic Agents

[0305] This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (*e.g.*, a NPHP4 or inversin modulating agent or mimetic, a NPHP4 or inversin specific antibody, or a NPHP4 or inversin binding partner) in an appropriate animal model (such as those described herein) to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be, *e.g.*, used for treatments of cystic kidney disease (*e.g.*, including, but not limited to, NPHP kidney disease).

IX. Pharmaceutical Compositions Containing NPHP4 Nucleic Acid, Peptides, and Analogs

[0306] The present invention further provides pharmaceutical compositions which may comprise all or portions of NPHP4 polynucleotide sequences, NPHP4 polypeptides, inhibitors or antagonists of NPHP4 bioactivity, including antibodies, alone or in combination with at least one other agent, such as a stabilizing compound, and may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water.

[0307] The methods of the present invention find use in treating diseases or altering physiological states characterized by mutant NPHP4 alleles (*e.g.*, NPHP type 4 kidney disease or RP). Peptides can be administered to the patient intravenously in a pharmaceutically acceptable carrier such as physiological saline. Standard methods for intracellular delivery of peptides can be used (*e.g.*, delivery via liposome). Such methods are well known to those of ordinary skill in the art. The formulations of this invention are useful for parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal. Therapeutic administration of a polypeptide intracellularly can also be accomplished using gene therapy as described above.

[0308] As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and interaction with other drugs being concurrently administered.

[0309] Accordingly, in some embodiments of the present invention, NPHP4 nucleotide and NPHP4 amino acid sequences can be administered to a patient alone, or in combination with other nucleotide sequences, drugs or hormones or in pharmaceutical compositions where it is mixed with excipient(s) or other pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert. In another embodiment of the present invention, NPHP4 polynucleotide sequences or NPHP4 amino acid sequences may be administered alone to individuals subject to or suffering from a disease.

[0310] Depending on the condition being treated, these pharmaceutical compositions may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Mack Publishing Co, Easton Pa.). Suitable routes may, for example, include oral or transmucosal administration; as well as parenteral delivery, including intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

[0311] For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. For tissue or cellular administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0312] In other embodiments, the pharmaceutical compositions of the present invention can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral or nasal ingestion by a patient to be treated.

[0313] Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. For example, an effective amount of NPHP4 may be that amount that suppresses apoptosis. Determination of effective amounts is well within the capability of those skilled in the art, especially in light of the disclosure provided herein.

[0314] In addition to the active ingredients these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

[0315] The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known (*e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes).

[0316] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0317] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, etc; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate.

[0318] Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, (*i.e.*, dosage).

[0319] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

[0320] Compositions comprising a compound of the invention formulated in a pharmaceutical acceptable carrier may be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. For polynucleotide or amino acid sequences of NPHP4, conditions indicated on the label may include treatment of condition related to apoptosis.

[0321] The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, *etc.* Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1 mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5 that is combined with buffer prior to use.

[0322] For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. Then, preferably, dosage can be formulated in animal models (particularly murine models) to achieve a desirable circulating concentration range that adjusts NPHP4 levels.

[0323] A therapeutically effective dose refers to that amount of NPHP4 that ameliorates symptoms of the disease state. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

[0324] The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state; age, weight, and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[0325] Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature (*See*, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212, all of which are herein incorporated by reference). Those skilled in the art will employ different formulations for NPHP4 than for the inhibitors of NPHP4. Administration to the bone marrow may necessitate delivery in a manner different from intravenous injections.

EXPERIMENTAL

[0326] The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

[0327] In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); °C (degrees Centigrade); U (units), mU (milliunits); min. (minutes); sec. (seconds); % (percent); kb (kilobase); bp (base pair); PCR (polymerase chain reaction); BSA (bovine serum albumin); Fisher (Fisher Scientific, Pittsburgh, PA); Sigma (Sigma Chemical Co., St. Louis, MO.); Promega (Promega Corp., Madison, WI); Perkin-Elmer (Perkin-Elmer/Applied Biosystems, Foster City, CA); Boehringer Mannheim (Boehringer Mannheim, Corp., Indianapolis, IN); Clonetech (Clonetech, Palo Alto, CA); Qiagen (Qiagen, Santa Clarita, CA); Stratagene (Stratagene Inc., La Jolla, CA); National Biosciences (National Biosciences Inc, Plymouth Minn.) and NEB (New England Biolabs, Beverly, MA), wt (wild-type); Ab (antibody); NPHP (nephronophthisis); SLS (Senior-Loken syndrome); RP (retinitis pigmentosa) and ESRD (end stage renal disease).

Example 1

A. Methods

Pedigree and Diagnosis

[0328] Blood samples and pedigrees were obtained following informed consent from patients with NPHP and their parents. Diagnostic criteria were (i) development of ESRD following a history of polyuria, polydipsia, and anemia; (ii) renal ultrasound compatible with NPHP. In all families with the exception of F461 the diagnosis of NPHP was confirmed by renal biopsy. ESRD developed within a range of 6–35 years with a median age of 22 years (Table 1). In SLS, the renal symptoms are associated with RP. Clinical data for SLS family F3 have been published previously (Polak *et al.*, Am J Ophthalmol 95:487–494 [1983]; Schuermann *et al.*, Am J Hum Genet 70:1240-1246 [2002]; herein incorporated by reference). All three affected siblings had RP suggestive of Leber amaurosis congenital. Ophthalmologic data for family F60 has been published (Fillastre *et al.*, Clin Nephrol 5:14-19 [1976]; herein incorporated by reference) and comprises: In J.C. (Fillastre *et al.* 1976, *supra*) amblyopia and rotary nystagmus with grossly impaired vision starting age 8 months, and on fundoscopy retino-choroidal atrophy surrounded by pigment. In individuals M.C.B. and M.M.B. there were abnormal ERG findings with diminished amplitude (Fillastre *et al.* 1976, *supra*).

Haplotype and Mutational Analysis

[0329] The "screening markers" used for haplotype analysis consisted of microsatellites markers *D1S2845, D1S2660, D1S2795, D1S2870, D1S2642, D1S214, D1S2663, D1S1612* (in pter to cen orientation) (Dib *et al.*, Nature 380:152 [1996]). Novel microsatellite markers were generated by searching for di-, tri-, and tetra-nucleotide repeats using the BLAST program on human genomic sequence in the interval between flanking markers *D1S2660* and *D1S2642*. Preparation of genomic DNA and haplotype analysis were performed as described previously (Schuermann *et al.* 2002, *supra*). Mutational analysis was performed using exon-flanking primers as described previously (Schuermann *et al.* 1996). Markers are shown in Table 2.

[0330] **Table 2.** Primer sequences (from 5' to 3') used in exon amplification for mutational analysis of *NPHP4*.

Exon	Forward Primer	Reverse Primer	Product Size (bp)
1	gtcggacatgcaaatcagg (SEQ ID NO:21)	aggctctggccaacactg (SEQ ID NO:51)	439
2	aagccttcaggattgctgtg (SEQ ID NO:22)	catccatctgttaactggaagc (SEQ ID NO:52)	319
3	acatggcctgccagtgac (SEQ ID NO:23)	cctggacccacaagtctgag (SEQ ID NO:53)	346
4	acgtgtaggaaggcggtctc (SEQ ID NO:24)	gacgagcagttaaaccaccatag (SEQ ID NO:54)	649
5	gaggcctccatgtgctttc (SEQ ID NO:25)	gctaaaggtggggaacactc (SEQ ID NO:55)	209
6	tgaccctcattgagaactgc (SEQ ID NO:26)	gtgccttcaaggtttcactg (SEQ ID NO:56)	217
7	ttgtgctctgtctgggagtc (SEQ ID NO:27)	catcagatgcggggtctc (SEQ ID NO:57)	439
8	ctcccccagggacttctg (SEQ ID NO:28)	cctgacatgcacaaatgacc (SEQ ID NO:58)	335
9	ttctgacagtggtcgacgtg (SEQ ID NO:29)	tgcccactacatttatcctcac (SEQ ID NO:59)	279
10	cactgttgatttcccctctc (SEQ ID NO:30)	gcaaacatatttgtgaacttttgc (SEQ ID NO:60)	343
11	ttcctggttggatcgttctg (SEQ ID NO:31)	cgacgattatcttacaaatgtgg (SEQ ID NO:61)	329
12	aggcctgtggagacctgac (SEQ ID NO:32)	ggggacagagggttttcttg (SEQ ID NO:62)	232
13	catgttgggagctttgtgg (SEQ ID NO:33)	gacaggcacagtgcaaaaac (SEQ ID NO:63)	262
14	atctgagcaccgttggttg (SEQ ID NO:34)	gggttcacaaggtccaacag (SEQ ID NO:64)	295
15	ggtttccacagggaggtg (SEQ ID NO:35)	aggtcagaacctcagcgaag (SEQ ID NO:65)	345
16	accatcccctatgcaaacac (SEQ ID NO:36)	gcactggtcaccgtatgattc (SEQ ID NO:66)	409
17	gaccagagctgaaatctctt (SEQ ID NO:37)	acgctggaagcgtgactc (SEQ ID NO:67)	315
18	cacagtggctttcctgctg (SEQ ID NO:38)	cgagggagcccacactctac (SEQ ID NO:68)	358
19	tgtggtgggttgatctgttt (SEQ ID NO:39)	cactgacagcaccacgaatg (SEQ ID NO:69)	332
20	ccctggtgtctgctcctg (SEQ ID NO:40)	gaggcagggaaaggatgtg (SEQ ID NO:70)	351
21	agcaatagccccttgtggag (SEQ ID NO:41)	tctcgggcagaattcgag (SEQ ID NO:71)	386
22	tctctcccactcctctgagc (SEQ ID NO:42)	agggacactggtggagactg (SEQ ID NO:72)	377
23	tggcagtggtgtctctaagc (SEQ ID NO:43)	aggaggggagagaaggacac (SEQ ID NO:73)	251
24	ttggcaacagtggagatacg (SEQ ID NO:44)	catgaggccatctgtcacc (SEQ ID NO:74)	342
25	tcttgctgagcacctgtgac	aggatacccgtggggaag	282

	(SEQ ID NO:45)	(SEQ ID NO:75)	
26	cactcgctgcgtgtattagt (SEQ ID NO:46)	caagcccactttcaatccac (SEQ ID NO:76)	268
27	ccttgttggcctctcgtg (SEQ ID NO:47)	ccagctgaatgcccactg (SEQ ID NO:77)	318
28	ggaaccacccatgaccttg (SEQ ID NO:48)	cagtggtccgagtcacagg (SEQ ID NO:78)	388
29	cagggaatacttggaggaag (SEQ ID NO:49)	gaggaactcgctcctaaatgc (SEQ ID NO:79)	310
30	gcagagaggttgctggtgag (SEQ ID NO:50)	accgggcttgtgctgtag (SEQ ID NO:80)	738

Northern Blot Analysis

[0331] A multiple tissue Northern blot with human adult poly(A)+ RNA (Clontech MTN7760-1) was hybridized with a *NPHP4* DNA probe of 584 bp, derived from exon 30 (nt 4141-4724; see FIG. 4) generated by PCR amplification of human genomic DNA. The probe was labeled with [^{32}P]dCTP using Random Primers DNA Labeling System (Invitrogen). Hybridization was carried out at 68°C using EXPRESSHYB solution (Clontech, Paolo Alto, CA). The final washing condition was 0.1 x SSC, 0.1% SDS at 50°C for 40 min.

B. Results

[0332] A gene locus (*NPHP4*) for NPHP type 4 was mapped by total genome search for linkage within a 2.1 Mb interval delimited by flanking markers *D1S2660* and *D1S2642* (Schuermann *et al.* 1996). To establish compatibility with linkage to *NPHP4* in further kindred, 20 NPHP families with multiple affected children or parental consanguinity, in whom no mutation was present in the *NPHP1* gene, were selected. In 8 families there was an association of NPHP with retinitis pigmentosa (RP). Haplotype analysis using 8 microsatellite markers covering the critical *NPHP4* region (Schuermann *et al.* 2002, *supra*; herein incorporated by reference) was compatible with linkage to *NPHP4* in 9 families, including 2 families with RP. To further refine the critical genetic interval of 2.1 Mb, high-resolution haplotype analysis was performed in these 9 families and the 7 families with linkage to *NPHP4* published previously (Schuermann *et al.*, 2002, *supra*). In 2 families (F3, F60) NPHP was associated with RP. Eight published (Dib *et al.* 1996, *supra*) and 38 newly generated microsatellite markers were used at an average marker density of 1 marker per 45 kb within the interval of flanking markers *D1S2660*and *D1S2642* (FIG. 1). Haplotype analysis, by the criterion of minimization of recombinants, clearly revealed erroneous inversion of sequence between markers *D1S2795* and *D1S244* in human genomic sequence data bases (www.ensembl.org).

[0333] Using high resolution haplotype data, the correct marker order at the *NPHP4* locus was established as pter-*D1S2660*-*D1S2795-D1S2633- D1S2870-D1S253-D1S2642-D1S214-D1S1612-D1S2663-D1S244*-cen (flanking markers to *NPHP4* underlined). A 22 kb sequence gap remaining in the interval *D1S2660 - D1S2795* was filled by use of CELERA human genomic sequence. In haplotype analysis, 3 consanguineous kindred yielded new key recombinants by the criterion of homozygosity by descent (Lander and Botstein, Science 236: 1567 [1987]) (FIG. 1). The *NPHP4* critical genetic interval was thus refined to <1.2 Mb within secure borders based on a large kindred, and in addition, to < 700 kb within suggestive borders based on 2 small families (FIG. 1, FIG. 2A, B).
Within the 700 kb critical interval for *NPHP4* there mapped 3 known genes (*KCNAB2*, *RPL22*, and *ICMT*), and 3 unknown genes (*Q9UFQ2, Q9UFR9,* and *Q96MP2*) (FIG. 2B). In addition, in the interval between *Q9UFQ2* and flanking marker *D1E19* (FIG. 2B) the program GENESCAN predicted approximately 40 non-annotated exons (www.ensembl.org). Mutational analysis was performed in affected individuals of the 16 families compatible with linkage to *NPHP4*, examining all 79 exons of the 3 known and 3 unknown genes by direct sequencing of the forward strands of exon-PCR products. While no mutations were detected in 5 of these genes, in *Q9UFQ2* detected 11 distinct mutations were detected in 8 of the 16 families with NPHP (Table 1). In families F3 and F60 NPHP is associated with RP. In the affected individuals from all 8 families, mutations were shown to segregate from both parents (Table 1). All of these mutations were absent from 92-96 healthy control individuals. Nine of the 11 mutations detected represent very likely loss-of-function mutations: 5 were STOP codon, 1 frame shift, and 3 were obligatory splice consensus mutations (Table 1 and FIGS. 2D and 6-16.). *Q9UFQ2* was thus identified as the gene causing NPHP type 4. The gene was termed *NPHP4* and the respective gene product was called "nephroretinin" for its role in nephronophthisis and retinitis pigmentosa. In the 5 consanguineous families F3, F30, F32, F60, and F622, all mutations occurred in the homozygous state and represented STOP codon mutations and one

frame shift mutation, truncating the protein in exons 18, 23, 11, 16, and 18, respectively (Table 1; FIG. 2D, E). In the 3 non-consanguineous families, 6 distinct compound heterozygous mutations were found. Four represented STOP codon or obligatory splice consensus mutations, truncating the gene product in exons 15, 16, 17, and 24. The missense mutations R848W and G754R affect amino acid residues conserved in mouse and cow. No mutations were detected in 8 families.

[0334] *NPHP4* expression studies by northern blot analysis revealed a 5.9 kb transcript strongly expressed in human skeletal muscle, weakly in kidney, and in 6 additional tissues studied (FIG. 3). Northern dot blot analysis confirmed a widespread expression pattern in human adult and fetal tissues including testis. This broad expression pattern, with strong expression in skeletal muscle and testis corresponds well with the expression pattern described for the *NPHP1* gene (Otto *et al.*, J. Am. Soc. Nephrol. 11:270 [2000]).

[0335] Human genomic sequence of *NPHP4* (KIAA0673) was assembled using the homo sapiens chromosome 1 working draft sequence segment NT_028054, which predicted 25 exons. Five additional 5' exons were identified using additional working draft sequence, the mRNA KIAA00673 and 57 human ESTs from the UniGene cluster Hs.106487. The genomic structure shown in FIG. 2C, D and FIG. 4 was confirmed by human/mouse total genomic sequence comparison. The *NPHP4* gene contains 30 exons encoding 1426 amino acids and extends over 130 kb, with splice sites that confirm to the canonical consensus gt-ag. An exception was found in intron 24, with gc-ag splicing, which occurs in 0.5% of mammalian splice sites (Burset *et al.*, Nuc. Acid. Res. 29:255 [2001]). A polymorphism is known to be present at the intron 20 splice acceptor (tg for ag). Presence of exon 20 is supported by 3 human EST clones. Ten different splice variants have been suggested for KIAA0673 (See e.g., the Internet web site of NCBI).

[0336] The *NPHP4* cDNA (FIG. 4) and deduced nephroretinin protein sequences were found to be novel, without any sequence similarity to known human cDNA or protein sequences. Therefore, *NPHP4* encodes a hitherto unknown protein. As shown for the *NPHP1* gene product nephrocystin (Hildebrandt *et al.*, Nature Genet. 17:149 [1997]; Otto *et al.*, J. Am. Soc. Nephrol. 11:270 [2000]), there was however strong sequence conservation for nephroretinin in evolution with 23% amino acid identity in a protein of *C. elegans* (FIG 5). Translated EST sequences also demonstrated evolutionary conservation in mouse, cow, pig, zebrafish, *Xenopus laevis*, *Ascaris suum*, and *Halocynthia roretzi.* Sequence identity of the murine homologue was 78% (FIG. 5). Analysis of nephroretinin amino acid sequence provided no signal sequence, conserved domains, or predicted transmembrane regions. In the N-terminal half there was a putative nuclear localization signal (NLS), a glutamate-rich (E-rich) and a proline-rich (P-rich) domain. The latter two have also been found in nephrocystin (Otto *et al.*, [2000], *supra*). No sequence similarity to nephrocystin was present. In addition, 2 serine rich (S-rich) sequences and a C-terminal endoplasmic reticulum membrane domain were found in human and murine nephroretinin sequences. Encoded by exons 15 and 16, there were was in nephroretinin a domain of unknown function (DUF339) with evolutionary conservation including prokaryotes and a 63 amino acid stretch with 30 % sequence identity to a gas vesicle protein of *Halobacterium salinarium* (FIG. 5).

Table 1 Clinical details and mutations detected in families with *NPHP4*

Family	Number of affec-teds	ESRD at age	Retinitis pigmen-tosa	Origin	Parental consan-guinity	Exon	Nucleotide change[b]	Effect on coding sequence	Segre-gation[c]
F3[a]	3	28 y, 30 y, 35 y	yes	Turkey	yes	18	C2335T	Q779X	hom
F24	2	ND	no	Germany	no	17	G2260A	G754R	P
						17	IVS16-1G>C	Splice site	M
F30[a]	3	18 y, 22 y, 22 y	no	Germany	yes	23	3272delT	STOP at codon L1121	hom
F32	2	19 y, 20 y	no	India	yes	11	TC1334-1335AA	F445X	hom
F60	4	6 y, 10 y, 17 y, 22 y	yes	France	yes	16	C1972T	R658X	hom
F444[a]	2	23 y, 33 y	no	Finland	no	15	IVS15+1 G>A	Splice site	M
						24	IVS24+1 G>A	Splice site	P
F461[a]	3	ND	no	France	no	16	C2044T	R682X	P
						19	C2542T	R848W	M
F622	2	8 y, 9 y	no	Afghanistan	yes	18	G2368T	E790X	hom

[a] In these 4 families linkage to *NPHP4* has been published previously (Schuermann et al. 2002).

[b] All mutations were absent from 92 - 96 healthy control subjects.

[c] M, maternal; P, paternal; hom, homozygous mutation inherited from both parents; ND, no data available.

Example 2

Mutations in INVS Cause NPHP2

[0337] Mutational analysis was performed on 16 exons of *INVS* in genomic DNA from nine affected individuals from seven different families with early onset of NPHP. One individual (from family A7) was included from the initial description (Gagnadoux *et al.*, Pediatr. Nephrol. 3, 50 [1989]) of infantile NPHP (individual 5) and two affected siblings (VII-1 and VII-3 in family A12) from the Bedouin kindred (Haider *et al.,* Am. J. Hum. Genet. 63, 1404 [1998]) in which the *NPHP2* locus was first mapped (Table 3). Nine distinct recessive mutations were detected in *INVS* (Table 3 and FIG. 15). In six individuals, both mutated alleles were detected. In individual A10, only one heterozygous mutation was found.

[0338] Mutations in *INVS* (nucleotide exchange and amino acid exchange) are shown (FIG 15a) together with sequence traces for mutated sequence (top) and sequence from healthy controls (bottom). Family numbers are given above boxes. If only one mutation is shown, it occurred in the homozygous state, except in individual A10, in whom only one mutation in the heterozygous state was detected. In individual 868, the 2742insA mutation is shown in the flipped version of the reverse strand. The exon structure of *INVS* is shown in FIG. 15b. Lines indicate relative positions and connect to mutations detected in *INVS.* Open and filled boxes represent *INVS* exons drawn relative to scale bar. Positions of start codon (ATG) at nucleotide +1 and of stop codon (TGA) are indicated. A representation of protein motifs drawn to scale parallel to exon structure is shown (FIG. 15c). Lines connect to point mutations detected, as shown in FIG 15a and 15d).

Example 3

Inversin associates with nephrocystin in HEK293T cells and mouse tissue

[0339] Myc-tagged nephrocystin (Myc–NPHP1) was coexpressed with N-terminally FLAG-tagged full-length inversin (FLAG–INV) or FLAG-tagged TRAF2 (FLAG–TRAF2) protein as a negative control. After immunoprecipitation with anti-FLAG antibody, coprecipitating nephrocystin was detected with nephrocystin-specific antiserum (FIG 26a, left panel). Protein expression levels in cellular lysates were controlled by immunoblotting using a nephrocystin antibody (FIG 26a, middle panel) or FLAG-specific and nephrocystin-specific antibodies (FIG 26a, right panel). Molecular weight markers are shown in kDa. Full-length nephrocystin was fused to the CH2 and CH3 domains of human IgG1 and precipitated with protein G sepharose beads. FLAG-tagged inversin specifically coprecipitated with nephrocystin but not with control protein (CH2 and CH3 domains of human IgG1 without nephrocystin fusion) as shown with FLAG-specific antibody (FIG 26b). FLAG-tagged nephrocystin or FLAG-tagged TRAF2 protein as a negative control was coexpressed with N-terminally Myc-tagged full-length inversin (Myc–INV). After immunoprecipitation with anti-FLAG antibody, coprecipitating inversin was detected with inversin-specific antiserum (FIG 26c, left and middle panels). Appropriate controls were also run (FIG 26c, right panel). A rabbit antiserum to a MBP–inversin fusion protein (amino acids 561–716 of mouse inversin) specifically recognized inversin (amino acids 1–716) expressed in HEK293T cells (FIG 26d, left panel) but not the FLAG-tagged control proteins podocin (FLAG–podocin), nephrocystin (FLAG–NPHP1) or PACS-1 (FLAG–PACS-1, amino acids 85–280) (FIG 26d, left panel). It also specifically recognized recombinant GST–inversin (amino acids 561–716) but not two other control GST fusion proteins (FIG 26d, lower panel). To show endogenous nephrocystin-inversin interaction *in vivo* in mouse kidney, half of mouse kidney tissue lysates was immunoprecipitated with a control antibody to hemagglutinin (anti-HA), and the other half was precipitated with anti-nephrocystin antisera. Immobilized inversin was detected with the inversin-specific antisera (FIG 26e, right upper panel). Precipitation of endogenous nephrocystin was confirmed by reprobing the blot for nephrocystin (FIG. 26e, right lower panel). Appropriate controls are also shown (FIG. 26e, eft panels).

Example 4

β-tubulin is a nephrocystin interaction partner

[0340] In order to identify nephrocystin-interacting proteins, HEK 293T cells were transfected with the FLAG-tagged control protein GFP or FLAG-tagged nephrocystin. Specific association of β-tubulin with nephrocystin was confirmed by immunoblotting of 2D gels using anti β - tubulin antibody (FIG. 27a). Several FLAG-tagged nephrocystin truncations were generated to analyze the interaction of nephrocystin with β-tubulin. Endogenous β-tubulin precipitated with transfected full-length nephrocystin but not with the control proteins GFP or TRAF2 (FIG 27b, upper panel). Expression of native β-tubulin in lysates is also shown (FIG 27b, middle panel). The membrane depicted in FIG 27b, middle panel, was reprobed with anti-FLAG antibody and shows that β-tubulin is still detected below the 62 kDa marker, confirming comparable expression levels of the FLAG-tagged proteins (FIG 27b, lower panel). The interaction was mapped to a region of nephrocystin involving amino acids 237–670 (FIG 27c, upper panel) with the expression levels of β-tubulin shown as a control (FIG 27c, bottom panel). The membrane was reprobed with anti-FLAG antibody to confirm expression of the FLAG-tagged proteins in the lysates (FIG 27c, lower panel). Endogenous β-tubulin coprecipitates with native nephrocystin in ciliated mCcd-K1 cells (FIG 27d).

Example 5

Inversin and nephrocystin colocalize with β-tubulin to cilia

[0341] Nephrocystin and β-tubulin-4 colocalize in primary cilia of MDCK cells (FIG 28a, upper and lower panels). Wild-type MDCK cells (clone II) were grown on coverslips at 100% confluence and cultivated for 7 d before the experiment to allow full polarization and cilia formation. Localization of nephrocystin was determined by immunofluorescence using nephrocystin-specific antibody with confocal images captured at the level of the apical membrane. Cells were costained with rabbit antibody to nephrocystin (FIG 28a, left panels) and mouse antibody to β-tubulin-4 (FIG 28a, middle panels) followed by the respective secondary antibodies. Specific localization of nephrocystin in primary cilia was confirmed by the use of blocking recombinant nephrocystin protein (FIG 28b). Inversin localizes to primary cilia in MDCK cells (FIG 28c). Localization of endogenous inversin was determined by immunofluorescence using inversin-specific antibody with confocal images captured at the level of the apical membrane. Cells were costained with mouse antibody to β-tubulin-4 and rabbit antibody to inversin followed by the respective secondary antibodies (FIG 28c, lower panel). In additional stainings, the antibody to β-tubulin-4 was omitted to reduce potential spectral overlap between the inversin and β-tubulin-4 signals (FIG 28c, upper panel). Partial colocalization of nephrocystin and inversin in primary cilia is observed (FIG 28d). Localization of nephrocystin was determined by immunofluorescence using nephrocystin-specific antibody with confocal images captured at the level of the apical membrane. Cells were costained with goat antibody to inversin (FIG 28d, left panel) and rabbit antibody to nephrocystin (FIG 28d, middle panel) followed by the respective secondary antibodies. Partial colocalization is shown (FIG 28d, right panel).

Example 6

Disruption of zebrafish invs function results in renal cyst formation

[0342] It was determined that embryos injected with a control, non-specific oligonucleotide have normal morphology (FIG 29a) whereas embryos injected with atgMO and spMO have a pronounced ventral axis curvature at 3 d.p.f. (combined totals for atgMO and spMO: 432 of 479 injected embryos; 90%) (FIG 29b). Coinjection of 100 pg mouse *Invs* mRNA with spMO completely rescued axis curvature defects (combined totals for atgMO and spMO: 363 of 381 mRNA+MO injected embryos were rescued; 95%).(FIG 29c). FIG 29d shows a histological section of a 2.5-d.p.f. control embryo pronephros showing the midline glomerulus (Gl), pronephric tubule (Pt) and pronephric duct (Pd). FIG 29e shows an atgMO-injected 3-d.p.f. embryo showing cystic dilatation of pronephric tubules and glomerulus (indicated with an asterisk) lined with squamous epithelium. FIG 29f shows that spMO similarly causes cystic maldevelopment of the pronephric tubules (marked with an asterisk). Molecular analysis of morpholino targeted *invs* splicing defects was performed. RT–PCR analysis of *invs* expression in 24-h.p.f. control injected embryos generates a 746-bp *invs* fragment encoding the C-terminal domain (FIG 29g, lane C, nucleotides 2,233–2,979 of GenBank AF465261; lane M, ϕX174 markers). spMO-injected embryos analyzed with the same RT–PCR primers generate a 189-bp RT–PCR product representing a C-terminal *invs* deletion allele (FIG 29g, lanes spMO; 24, 48 and 72 h.p.f.). Some recovery of wild-type (WT) mRNA is observed at 72 h.p.f. RT–PCR of

ACTB mRNA on the same RNA samples as in FIG 29g shows no effect of morpholino injection at any time point (FIG 29h). FIG 29i diagrams the effect of spMO on *invs* mRNA processing. Preventing normal splicing in the IQ2 domain recruits a cryptic splice donor in upstream *invs* coding sequence, the resulting out-of-frame fusion generates a C-terminally truncated *invs* mRNA at amino acid 696 with an altered 21 amino acid C terminus (FIG 29i). Rescue of normal morphology by coinjected spMO and mouse *Invs* mRNA shows a normal pronephric duct structure (Pt) (FIG 29j) as compared to the absence of any effect when the *Invs* mRNA was injected alone.

Table 3:

Family (individual)	Ethnic origin	Nucleotide alteration(s)[a]	Alteration(s) in coding sequence	Exon, segregation[b]	Parental consanguinity	Renal cysts	Renal biopsy	Age at ESRD[c]	*Situs inversus* (other symptoms)[d]
A6	France	C2695T	R899X	13, het[e]	–	–	+	<2 y	–
		1453delC	Q485fsX509	9, het[e]					
A8	Turkey	C1807T	R603X	12, hom[e]	+	–	+	14 mo	+ (VSD[f])
A9	France	C1186T	R396X	8, het[e]	–	+	+	<2 y	–
		C1445G	P482R	9, het P					
A10[g]	France	2908delG	E970fsX971	14, het M	–	+	+	12 mo	–
A12 (VII-1, VII-3)	Israel	C2719T	R907X	13, hom M,P	+	+	(+, +)	(30 mo, 30 mo)	–, – (HT, HT)
868 (II-1, II-2)	USA	C2719T	R907X	13, het M	–	–[h]	(+, +)	(5 y, 4 y)	–, – (HT, HT)
		2747insA	K916fsX1002	13, het P					
A7	Portugal	T1478C	L493S	10, hom[e]	+	ND	+	5 y	– (HT)

[a]All mutations were absent from at least 100 healthy control subjects. [b]M, maternal; P, paternal; het, heterozygous; hom, homozygous mutation inherited from both parents; ND, no data available. [c]ESRD, end stage renal disease; mo, months. [d]HT, arterial hypertension. [e]Parent(s) not available for mutational analysis. [f]VSD, cardiac ventricular septal defect. [g]Only one mutation was detected in this individual. [h]Hyperechogenicity noted as sign of incipient microcysts.

[0343] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in molecular biology, genetics, or related fields are intended to be within the scope of the following claims.

CLAIMS

What is claimed is:

1. An isolated and purified nucleic acid comprising a sequence encoding a protein selected from the group consisting of SEQ ID NOs: 2, 6, 8, 10, 12, 14, 16, 18, and 20.

2. The nucleic acid sequence of Claim 1, wherein said sequence is operably linked to a heterologous promoter.

3. The nucleic acid sequence of Claim 1, wherein said sequence is contained within a vector.

4. The nucleic acid sequence of Claim 3, wherein said vector is within a host cell.

5. An isolated and purified nucleic acid sequence that hybridizes under conditions of low stringency to a nucleic acid selected from the group consisting of SEQ ID NOs: 1, 5, 7, 9, 11, 13, 15, 17, and 19.

6. A vector comprising the nucleic acid sequence of Claim 5.

7. A host cell comprising the vector of Claim 6.

8. The host cell of Claim 7, wherein said host cell is located in an organism, wherein said organism is a non-human animal.

9. A polypeptide encoded by a nucleic acid selected from the group consisting of SEQ ID NOs:1 and variants thereof that are at least 80% identical to SEQ ID NOs: 1 5, 7, 9, 11, 13, 15, 17, and 19.

10. The protein of Claim 9, wherein said protein is at least 90% identical to SEQ ID NOs: 1, 5, 7, 9, 11, 13, 15, 17, and 19.

11. The protein of Claim 9, wherein said protein is at least 95% identical to SEQ ID NOs: 1, 5, 7, 9, 11, 13, 15, 17, and 19.

12. A composition comprising a nucleic acid that inhibits the binding of at least a portion of a nucleic acid selected from the group consisting of SEQ ID NOs:1, 5, 7, 9, 11, 13, 15, 17, and 19 to their complementary sequences.

13. A polynucleotide sequence comprising at least fifteen nucleotides capable of hybridizing under stringent conditions to the isolated nucleotide sequence of Claim 5.

14. A composition comprising a variant nephroretinin polypeptide, wherein said polypeptide comprises a C-terminal truncation of SEQ ID NO:2.

15. The composition of Claim 14, wherein said variant nephroretinin polypeptide is selected from the group consisting of SEQ ID NOs: 6, 10, 12, 14, 16, and 20.

16. The composition of Claim 14, wherein the presence of said variant polypeptide in a subject is indicative of nephronophthisis type 4 kidney disease in said subject.

17. A method for detection of a variant nephroretinin polypeptide in a subject, comprising:

a) providing a biological sample from a subject, wherein said biological sample comprises a nephroretinin polypeptide; and

b) detecting the presence or absence of a variant nephroretinin polypeptide in said biological sample.

18. The method of Claim 17, wherein said variant nephroretinin polypeptide is a C-terminal truncation of SEQ ID NO:2.

19. The method of Claim 18, wherein said variant nephroretinin polypeptide is selected from the group consisting of SEQ ID NOs: 6, 10, 12, 14, 16, and 20.

20. The method of Claim 17, wherein the presence of said variant nephroretinin polypeptide is indicative of nephronophthisis type 4 kidney disease in said subject.

21. The method of Claim 17, wherein said biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample.

22. The method of Claim 17, wherein said subject is selected from the group consisting of an embryo, a fetus, a newborn animal, and a young animal.

23. The method of Claim 22, wherein said animal is a human.

24. The method of Claim 17, wherein said detecting comprises differential antibody binding.

25. The method of Claim 17, wherein said detecting comprises a gel-free truncation test.

26. The method of Claim 17, wherein said detection comprises a Western blot.

27. A kit comprising a reagent for detecting the presence or absence of a variant nephroretinin polypeptide in a biological sample.

28. The kit of Claim 27, further comprising instruction for using said kit for said detecting the presence or absence of a variant nephroretinin polypeptide in a biological sample.

29. The kit of Claim 28, wherein said instructions comprise instructions required by the U.S. Food and Drug Agency for *in vitro* diagnostic kits.

30. The kit of Claim 27, further comprising instructions for diagnosing nephronophthisis in said subject based on the presence or absence of said variant nephroretinin polypeptide.

31. The kit of Claim 30, wherein said nephronophthisis is nephronophthisis type 4.

32. The kit of Claim 27, wherein said reagent is one or more antibodies.

33. The kit of Claim 32, wherein said antibodies comprise a first antibody that specifically binds to the C-terminus of said nephroretinin polypeptide and a second antibody that specifically binds to the N-terminus of said nephroretinin polypeptide.

34. The kit of Claim 27, wherein said reagents comprise reagents for performing a gel-free truncation test.

35. The kit of Claim 27, wherein said variant nephroretinin polypeptide is a C-terminal truncation of SEQ ID NO:2.

36. The kit of Claim 27, wherein said variant nephroretinin polypeptide is selected from the group consisting of SEQ ID NOs: 6, 10, 12, 14, 16, and 20.

37. The kit of Claim 27, wherein said biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample.

38. A computer readable medium encoding a representation of the nucleic acid sequence of Claim 1.

39. A computer readable medium encoding a representation of the polypeptide sequence of Claim 9.

40. A method for detection of a variant inversin polypeptide in a subject, comprising:

a) providing a biological sample from a subject, wherein said biological sample comprises an inversin polypeptide; and

b) detecting the presence or absence of a variant inversin polypeptide in said biological sample.

41. The method of Claim 40, wherein said variant inversin polypeptide is a C-terminal truncation of SEQ ID NO:22.

42. The method of Claim 41, wherein said variant inversin polypeptide is selected from the group consisting of SEQ ID NOs: 24, 26, 28, 30, 34, 36, 38 and 40.

43. The method of Claim 40, wherein the presence of said variant inversin polypeptide is indicative of nephronophthisis type 2 kidney disease in said subject.

44. The method of Claim 40, wherein said biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample.

45. The method of Claim 40, wherein said subject is selected from the group consisting of an embryo, a fetus, a newborn animal, and a young animal.

46. The method of Claim 45, wherein said animal is a human.

47. The method of Claim 40, wherein said detecting comprises differential antibody binding.

48. The method of Claim 40, wherein said detecting comprises a gel-free truncation test.

49. The method of Claim 40, wherein said detection comprises a Western blot.

50. A kit comprising a reagent for detecting the presence or absence of a variant inversin polypeptide in a biological sample.

51. The kit of Claim 50 further comprising reagents for detecting the presence or absence of a variant nephroretinin polypeptide or a variant nephrocystin-3 polypeptide in a biological sample.

52. The kit of Claim 50, further comprising instruction for using said kit for said detecting the presence or absence of a variant inversin polypeptide in a biological sample.

53. The kit of Claim 52, wherein said instructions comprise instructions required by the U.S. Food and Drug Agency for *in vitro* diagnostic kits.

54. The kit of Claim 50, further comprising instructions for diagnosing nephronophthisis in said subject based on the presence or absence of said variant inversin polypeptide.

55. The kit of Claim 51 further comprising instructions for diagnosing nephronophthisis in said subject based on the presence or absence of said variant inversin polypeptide, said variant nephroretinin polypeptide, or said variant nephrocystin-3 polypeptide.

56. The kit of Claim 54, wherein said nephronophthisis is nephronophthisis type 2.

57. The kit of Claim 55, wherein said nephronophthisis is nephronophthisis type 2, nephronophthisis type 4, or nephronophthisis type 3.

58. The kit of Claim 50, wherein said reagent is one or more antibodies.

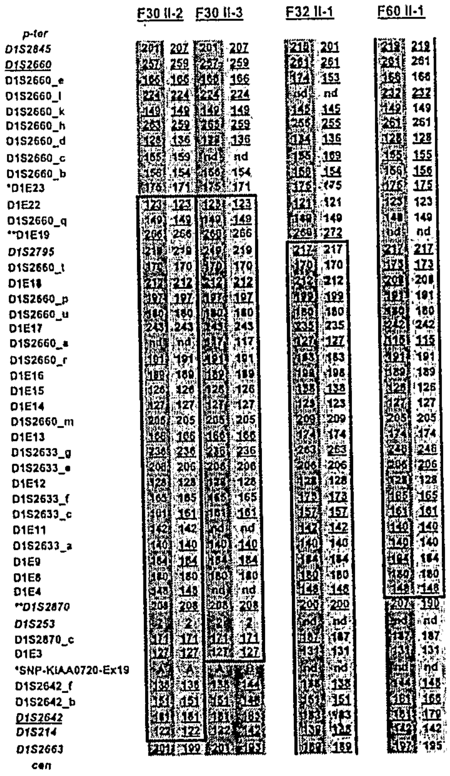
59. The kit of Claim 58, wherein said antibodies comprise a first antibody that specifically binds to the C-terminus of said inversin polypeptide and a second antibody that specifically binds to the N-terminus of said inversin polypeptide.

60. The kit of Claim 50, wherein said reagents comprise reagents for performing a gel-free truncation test.

61. The kit of Claim 50, wherein said variant inversin polypeptide is a C-terminal truncation of SEQ ID NO:22.

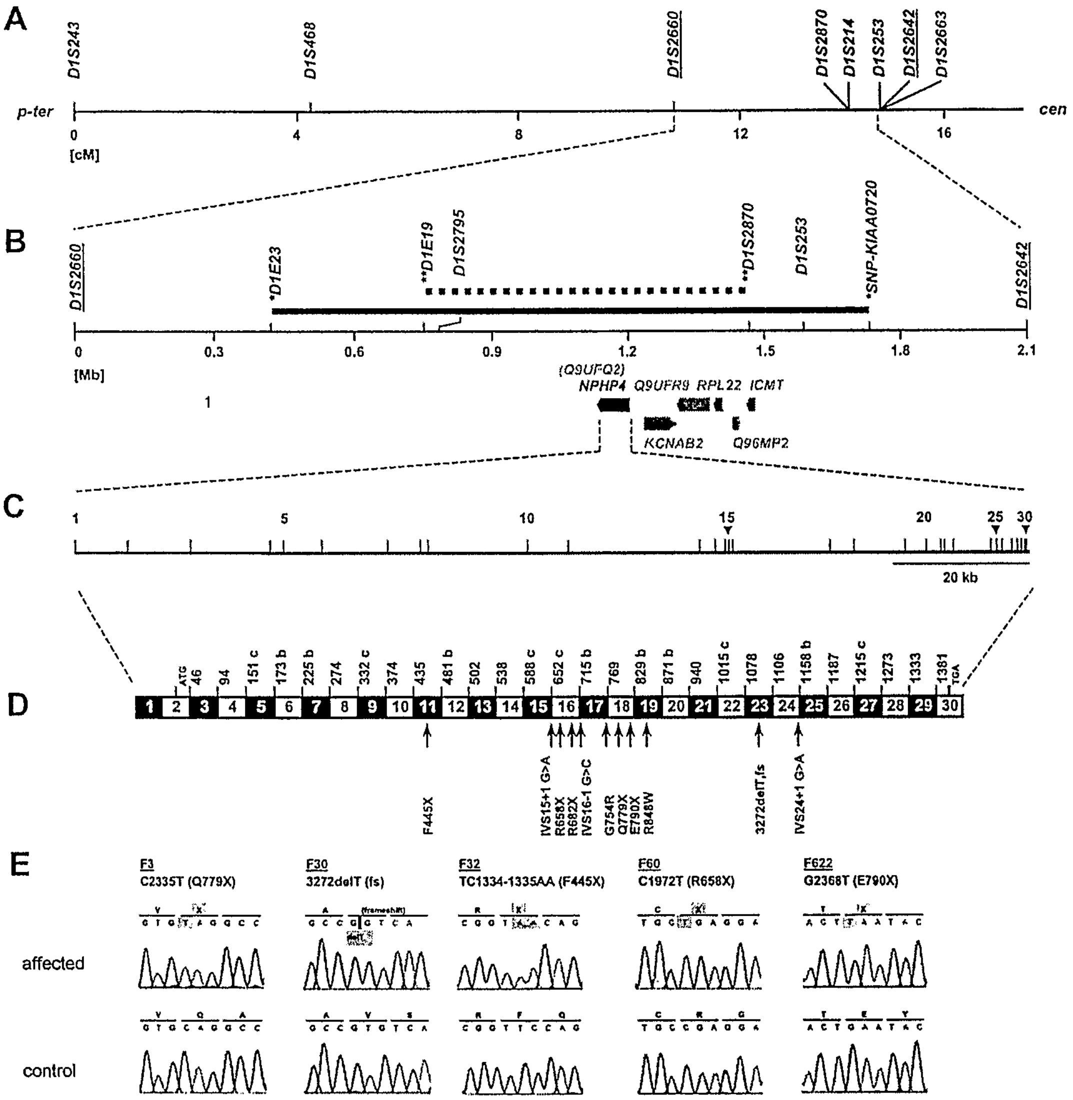
62. The kit of Claim 50, wherein said variant inversin polypeptide is selected from the group consisting of SEQ ID NOs: 24, 26, 28, 30, 34, 36, 38 and 40.

63. The kit of Claim 50, wherein said biological sample is selected from the group consisting of a blood sample, a tissue sample, a urine sample, and an amniotic fluid sample.



	F30 II-2		F30 II-3		F32 II-1		F60 II-1	
p-ter								
D1S2845	201	207	201	207	218	201	219	219
D1S2660	257	259	257	259	261	261	261	261
D1S2660_e	166	166	166	166	174	153	166	166
D1S2660_l	224	224	224	224	nd	nd	232	232
D1S2660_k	149	149	149	149	145	145	149	149
D1S2660_h	263	259	263	259	255	255	261	261
D1S2660_d	128	136	128	136	134	136	128	128
D1S2660_c	165	159	nd	nd	155	169	155	155
D1S2660_b	166	154	166	154	168	154	166	156
*D1E23	176	171	175	171	176	175	176	175
D1E22	123	123	123	123	121	121	123	123
D1S2660_q	149	149	149	149	149	149	149	149
**D1E19	266	266	266	266	266	272	nd	nd
D1S2795	219	219	219	219	217	217	217	217
D1S2660_t	170	170	170	170	170	170	173	173
D1E18	212	212	212	212	212	212	208	208
D1S2660_p	197	197	197	197	199	199	191	191
D1S2660_u	180	180	180	180	180	180	180	180
D1E17	243	243	243	243	235	235	242	242
D1S2660_a	nd	nd	117	117	127	127	115	115
D1S2660_r	191	191	191	191	183	183	191	191
D1E16	189	189	189	189	198	198	189	189
D1E15	126	126	126	126	138	138	126	126
D1E14	127	127	127	127	123	123	127	127
D1S2660_m	205	205	205	205	209	209	205	205
D1E13	166	166	166	166	174	174	174	174
D1S2633_g	236	236	236	236	263	263	246	246
D1S2633_e	206	206	206	206	206	206	206	206
D1E12	128	128	128	128	128	128	128	128
D1S2633_f	165	165	165	165	173	173	165	165
D1S2633_c	161	161	161	161	157	157	161	161
D1E11	142	142	nd	nd	142	142	140	140
D1S2633_a	140	140	140	140	140	140	140	140
D1E9	164	164	164	164	164	164	164	164
D1E8	180	180	180	180	180	180	180	180
D1E4	148	148	nd	nd	148	148	148	148
***D1S2870*	208	208	208	208	200	200	207	190
D1S253	2	2	2	2	nd	nd	nd	nd
D1S2870_c	171	171	171	171	187	187	187	187
D1E3	127	127	127	127	131	131	131	131
*SNP-KIAA0720-Ex19	A	A	A	B	nd	nd	nd	nd
D1S2642_f	138	138	138	144	138	138	144	148
D1S2642_b	151	151	151	148	151	151	161	166
D1S2642	161	161	161	183	183	183	161	179
D1S214	122	122	122	142	139	138	142	142
D1S2663	201	199	201	193	189	189	197	195
cen								

Fig. 1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D1S243
D1S468
D1S2660
D1S2870
D1S214
D1S253
D1S2642
D1S2663
p-ter
cen
0
4
8
12
16
[cM]
B
D1S2660
*D1E23
**D1E19
D1S2795
**D1S2870
D1S253
*SNP-KIAA0720
D1S2642
0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
[Mb]
(Q9UFQ2)
NPHP4
Q9UFR9
RPL22
ICMT
1
KCNAB2
Q96MP2
C
1
5
10
15
20
25
30
20 kb
D
ATG
46
94
151 c
173 b
225 b
274
332 c
374
435
481 b
502
538
588 c
652 c
715 b
769
829 b
871 b
940
1015 c
1078
1106
1158 b
1187
1215 c
1273
1333
1381
TGA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
F445X
IVS15+1 G>A
R658X
R682X
IVS16-1 G>C
G754R
Q779X
E790X
R848W
3272delT,fs
IVS24+1 G>A
E
F3
C2335T (Q779X)
F30
3272delT (fs)
F32
TC1334-1335AA (F445X)
F60
C1972T (R658X)
F622
G2368T (E790X)
(frameshift)
delT
affected
control

Fig. 2

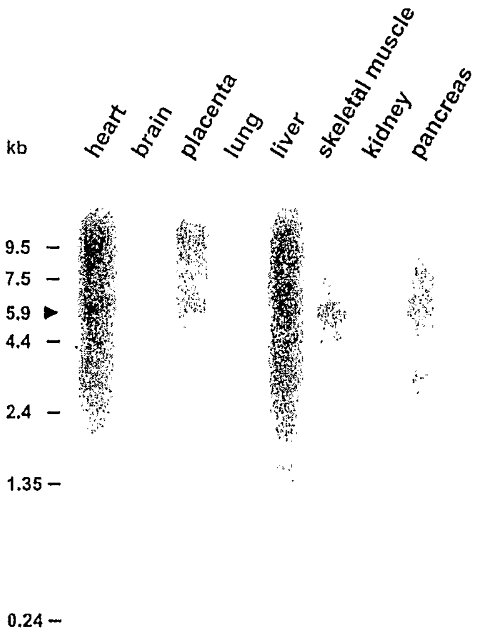

kb
heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
9.5
7.5
5.9
4.4
2.4
1.35
0.24

Fig. 3

Figure 4

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC        Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20    Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60    Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120   Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480   Exon 5
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600   Exon 6
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240   Exon 7

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900   Exon 8
281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300
```

```
     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F    320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R    340   Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L    360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L    380

     ---------|---------|---------|---------|---------|---------|   Exon 10
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A    400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C    420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S    440   Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E    460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S    480

     ---------|---------|---------|---------|---------|---------|   Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P    500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560   Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A    520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F    540

     ---------|---------|---------|---------|---------|---------|   Exon 14
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L    560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I    580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L    600   Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V    620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S    640

     ---------|---------|---------|---------|---------|---------|
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T    660

                                                                      Exon 16
```

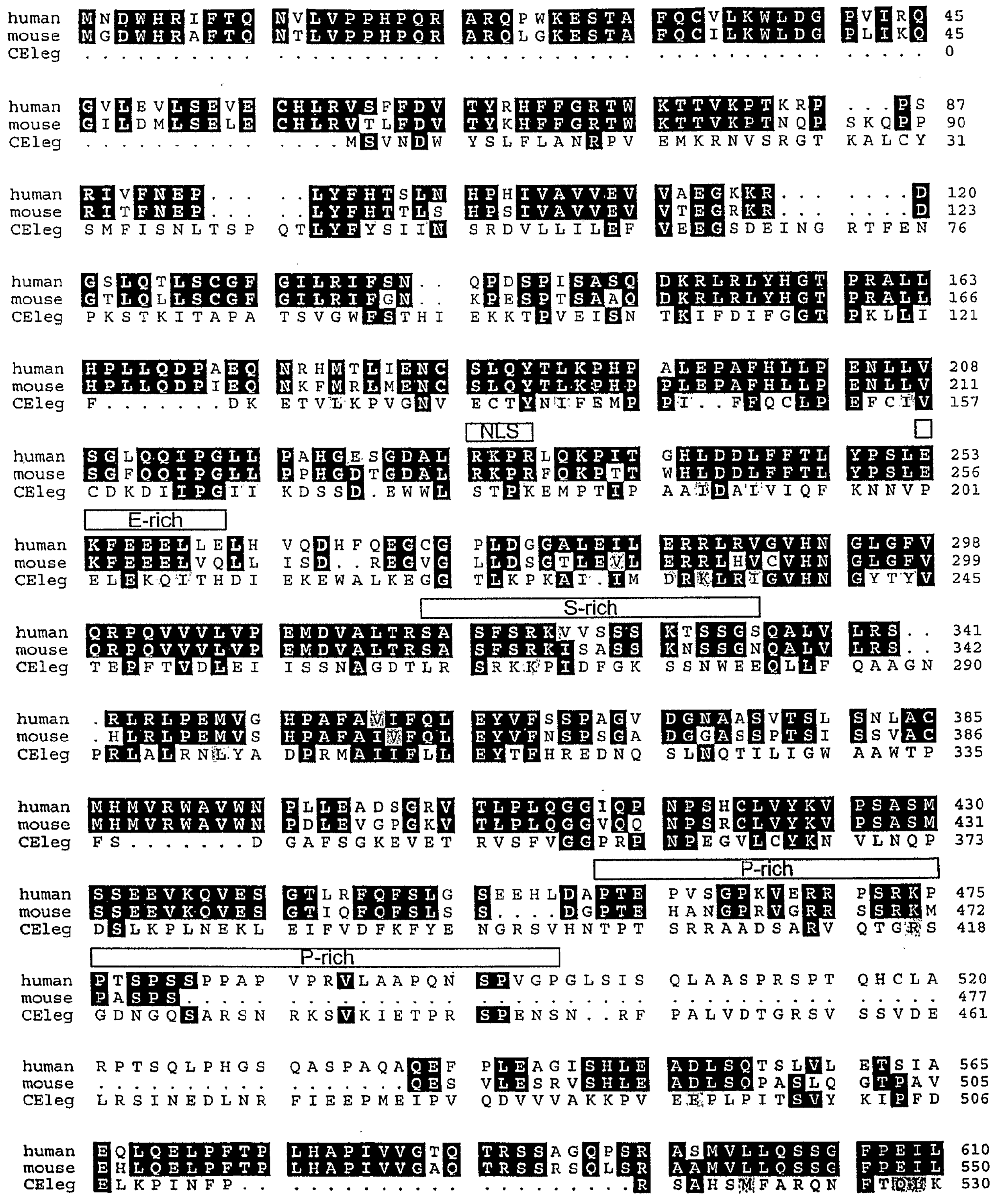
```
     ----------|----------|----------|----------|----------|----------|
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661  S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T   680

     ----------|----------|----------|----------|----------|----------|
2041 CCACGACTGCAGCTGGTCCAGCTGGATGAGGCCGGCCAGCCCAGCTCTGGCGCCCTGACC 2100
 681  P  R  L  Q  L  V  Q  L  D  E  A  G  Q  P  S  S  G  A  L  T   700

     ----------|----------|----------|----------|----------|----------|
2101 CACATCCTCGTGCCTGTGAGCAGAGATGGCACCTTTGATGCTGGGTCTCCTGGCTTCCAG 2160
 701  H  I  L  V  P  V  S  R  D  G  T  F  D  A  G  S  P  G  F  Q   720

     ----------|----------|----------|----------|----------|----------|
2161 CTGAGGTACATGGTGGGCCCTGGGTTCCTGAAGCCAGGTGAGCGGCGCTGCTTTGCCCGC 2220   Exon 17
 721  L  R  Y  M  V  G  P  G  F  L  K  P  G  E  R  R  C  F  A  R   740

     ----------|----------|----------|----------|----------|----------|
2221 TACCTGGCCGTGCAGACCCTGCAGATTGACGTCTGGGACGGAGACTCCCTGCTGCTCATC 2280
 741  Y  L  A  V  Q  T  L  Q  I  D  V  W  D  G  D  S  L  L  L  I   760

     ----------|----------|----------|----------|----------|----------|
2281 GGATCTGCTGCCGTCCAGATGAAGCATCTCCTCCGCCAAGGCCGGCCGGCTGTGCAGGCC 2340
 761  G  S  A  A  V  Q  M  K  H  L  L  R  Q  G  R  P  A  V  Q  A   780

     ----------|----------|----------|----------|----------|----------|   Exon 18
2341 TCCCACGAGCTTGAGGTCGTGGCAACTGAATACGAGCAGGACAACATGGTGGTGAGTGGA 2400
 781  S  H  E  L  E  V  V  A  T  E  Y  E  Q  D  N  M  V  V  S  G   800

     ----------|----------|----------|----------|----------|----------|
2401 GACATGCTGGGGTTTGGCCGCGTCAAGCCCATCGGCGTCCACTCGGTGGTGAAGGGCCGG 2460
 801  D  M  L  G  F  G  R  V  K  P  I  G  V  H  S  V  V  K  G  R   820

     ----------|----------|----------|----------|----------|----------|
2461 CTGCACCTGACTTTGGCCAACGTGGGTCACCCGTGTGAACAGAAAGTGAGAGGTTGTAGC 2520
 821  L  H  L  T  L  A  N  V  G  H  P  C  E  Q  K  V  R  G  C  S   840

     ----------|----------|----------|----------|----------|----------|   Exon 19
2521 ACATTGCCACCGTCCAGATCTCGGGTCATCTCAAACGATGGAGCCAGCCGCTTCTCTGGA 2580
 841  T  L  P  P  S  R  S  R  V  I  S  N  D  G  A  S  R  F  S  G   860

     ----------|----------|----------|----------|----------|----------|
2581 GGCAGCCTCCTCACGACTGGAAGCTCAAGGCGAAAACACGTGGTGCAAGCACAGAAGCTG 2640
 861  G  S  L  L  T  T  G  S  S  R  R  K  H  V  V  Q  A  Q  K  L   880

     ----------|----------|----------|----------|----------|----------|
2641 GCGGACGTGGACAGTGAGCTGGCTGCCATGCTACTGACCCATGCCCGGCAGGGCAAGGGG 2700   Exon 20
 881  A  D  V  D  S  E  L  A  A  M  L  L  T  H  A  R  Q  G  K  G   900

     ----------|----------|----------|----------|----------|----------|
2701 CCCCAGGACGTCAGCCGCGAGTCGGATGCCACCCGCAGGCGTAAGCTGGAGCGGATGAGG 2760
 901  P  Q  D  V  S  R  E  S  D  A  T  R  R  R  K  L  E  R  M  R   920

     ----------|----------|----------|----------|----------|----------|
2761 TCTGTGCGCCTGCAGGAGGCCGGGGGAGACTTGGGCCGGCGCGGGACGAGCGTGTTGGCG 2820
 921  S  V  R  L  Q  E  A  G  G  D  L  G  R  R  G  T  S  V  L  A   940

     ----------|----------|----------|----------|----------|----------|
2821 CAGCAGAGCGTCCGCACACAGCACTTGCGGGACCTACAGGTCATCGCCGCCTACCGGGAA 2880   Exon 21
 941  Q  Q  S  V  R  T  Q  H  L  R  D  L  Q  V  I  A  A  Y  R  E   960

     ----------|----------|----------|----------|----------|----------|
2881 CGCACGAAGGCCGAGAGCATCGCCAGCCTGCTGAGCCTGGCCATCACCACGGAGCACACG 2940
 961  R  T  K  A  E  S  I  A  S  L  L  S  L  A  I  T  T  E  H  T   980

     ----------|----------|----------|----------|----------|----------|
2941 CTCCACGCCACGCTGGGGGTCGCCGAGTTCTTTGAGTTTGTGCTTAAGAACCCCCACAAC 3000
 981  L  H  A  T  L  G  V  A  E  F  F  E  F  V  L  K  N  P  H  N  1000

     ----------|----------|----------|----------|----------|----------|
3001 ACACAGCACACGGTGACTGTGGAGATCGACAACCCCGAGCTCAGCGTCATCGTGGACAGT 3060
```

```
1001 T  Q  H  T  V  T  V  E  I  D  N  P  E  L  S  V  I  V  D  S   1020

     ---------|---------|---------|---------|---------|---------|      Exon 22
3061 CAGGAGTGGAGGGACTTCAAGGGTGCTGCTGGCCTGCACACACCGGTGGAGGAGGACATG 3120
1021 Q  E  W  R  D  F  K  G  A  A  G  L  H  T  P  V  E  E  D  M   1040

     ---------|---------|---------|---------|---------|---------|
3121 TTCCACCTGCGTGGCAGCCTGGCCCCCCAGCTCTACCTGCGCCCCCACGAGACCGCCCAC 3180
1041 F  H  L  R  G  S  L  A  P  Q  L  Y  L  R  P  H  E  T  A  H   1060

     ---------|---------|---------|---------|---------|---------|
3181 GTCCCCTTCAAGTTCCAGAGCTTCTCTGCAGGGCAGCTGGCCATGGTGCAGGCCTCTCCT 3240
1061 V  P  F  K  F  Q  S  F  S  A  G  Q  L  A  M  V  Q  A  S  P   1080
                                                                       Exon 23
     ---------|---------|---------|---------|---------|---------|
3241 GGGTTGAGCAACGAGAAGGGCATGGACGCCGTGTCACCTTGGAAGTCCAGCGCAGTGCCC 3300
1081 G  L  S  N  E  K  G  M  D  A  V  S  P  W  K  S  S  A  V  P   1100

     ---------|---------|---------|---------|---------|---------|
3301 ACTAAACACGCCAAGGTCTTGTTCCGAGCGAGTGGTGGCAAGCCCATCGCCGTGCTCTGC 3360
1101 T  K  H  A  K  V  L  F  R  A  S  G  G  K  P  I  A  V  L  C   1120  Exon 24

     ---------|---------|---------|---------|---------|---------|
3361 CTGACTGTGGAGCTGCAGCCCCACGTGGTGGACCAGGTCTTCCGCTTCTATCACCCGGAG 3420
1121 L  T  V  E  L  Q  P  H  V  V  D  Q  V  F  R  F  Y  H  P  E   1140

     ---------|---------|---------|---------|---------|---------|
3421 CTCTCCTTCCTGAAGAAGGCCATCCGCCTGCCGCCCTGGCACACATTTCCAGGTGCTCCG 3480
1141 L  S  F  L  K  K  A  I  R  L  P  P  W  H  T  F  P  G  A  P   1160  Exon 25

     ---------|---------|---------|---------|---------|---------|
3481 GTGGGAATGCTTGGTGAGGACCCCCCAGTCCATGTTCGCTGCAGCGACCCGAACGTCATC 3540
1161 V  G  M  L  G  E  D  P  P  V  H  V  R  C  S  D  P  N  V  I   1180

     ---------|---------|---------|---------|---------|---------|
3541 TGTGAGACCCAGAATGTGGGCCCCGGGGAACCACGGGACATATTTCTGAAGGTGGCCAGT 3600
1181 C  E  T  Q  N  V  G  P  G  E  P  R  D  I  F  L  K  V  A  S   1200  Exon 26

     ---------|---------|---------|---------|---------|---------|
3601 GGTCCAAGCCCGGAGATCAAAGACTTCTTTGTCATCATTTACTCGGATCGCTGGCTGGCG 3660
1201 G  P  S  P  E  I  K  D  F  F  V  I  I  Y  S  D  R  W  L  A   1220

     ---------|---------|---------|---------|---------|---------|
3661 ACACCCACACAGACGTGGCAGGTCTACCTCCACTCCCTGCAGCGCGTGGATGTCTCCTGC 3720
1221 T  P  T  Q  T  W  Q  V  Y  L  H  S  L  Q  R  V  D  V  S  C   1240  Exon 27

     ---------|---------|---------|---------|---------|---------|
3721 GTCGCAGGCCAGCTGACCCGCCTGTCCCTTGTCCTTCGGGGGACACAGACAGTGAGGAAA 3780
1241 V  A  G  Q  L  T  R  L  S  L  V  L  R  G  T  Q  T  V  R  K   1260

     ---------|---------|---------|---------|---------|---------|
3781 GTGAGAGCTTTCACCTCTCATCCCCAGGAGCTGAAGACAGACCCCAAAGGTGTCTTCGTG 3840
1261 V  R  A  F  T  S  H  P  Q  E  L  K  T  D  P  K  G  V  F  V   1280
                                                                       Exon 28
     ---------|---------|---------|---------|---------|---------|
3841 CTGCCGCCTCGTGGGGTGCAGGACCTGCATGTTGGCGTGAGGCCCCTTAGGGCCGGCAGC 3900
1281 L  P  P  R  G  V  Q  D  L  H  V  G  V  R  P  L  R  A  G  S   1300

     ---------|---------|---------|---------|---------|---------|
3901 CGCTTTGTCCATCTCAACCTGGTGGACGTGGATTGCCACCAGCTGGTGGCCTCCTGGCTC 3960
1301 R  F  V  H  L  N  L  V  D  V  D  C  H  Q  L  V  A  S  W  L   1320

     ---------|---------|---------|---------|---------|---------|
3961 GTGTGCCTCTGCTGCCGCCAGCCGCTCATCTCCAAGGCCTTTGAGATCATGTTGGCTGCG 4020
1321 V  C  L  C  C  R  Q  P  L  I  S  K  A  F  E  I  M  L  A  A   1340

     ---------|---------|---------|---------|---------|---------|
4021 GGCGAAGGGAAGGGTGTCAACAAGAGGATCACCTACACCAACCCCTACCCCTCCCGGAGG 4080  Exon 29
1341 G  E  G  K  G  V  N  K  R  I  T  Y  T  N  P  Y  P  S  R  R   1360
     ---------|---------|---------|---------|---------|---------|
```

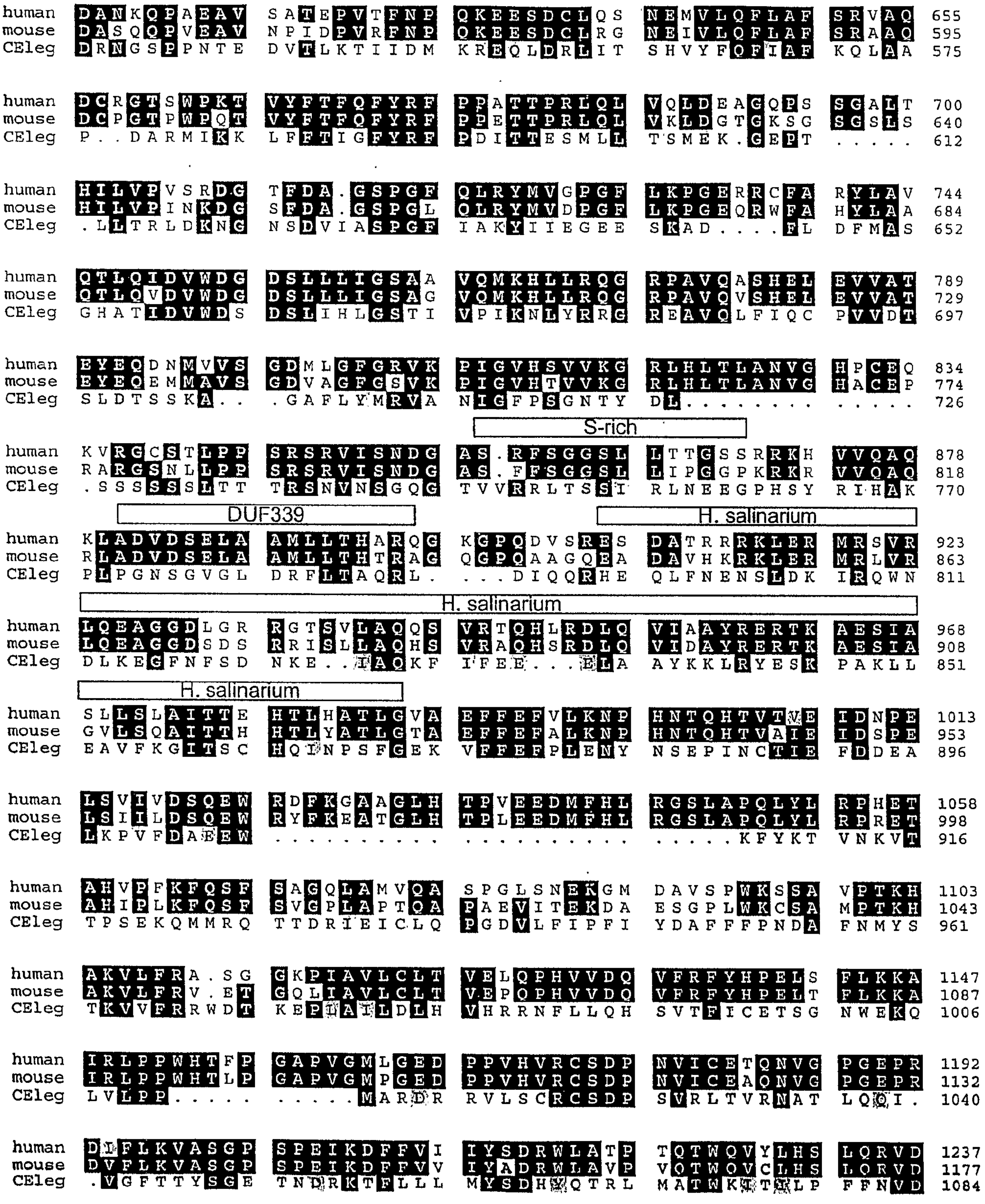
```
4081 ACATTCCACCTGCACAGCGACCACCCGGAGCTGCTGCGGTTCAGAGAGGACTCCTTCCAG 4140
1361 T  F  H  L  H  S  D  H  P  E  L  L  R  F  R  E  D  S  F  Q   1380

     ---------|---------|---------|---------|---------|---------|
4141 GTCGGGGGTGGAGAGACCTACACCATCGGCTTGCAGTTTGCGCCTAGTCAGAGAGTGGGT 4200
1381 V  G  G  G  E  T  Y  T  I  G  L  Q  F  A  P  S  Q  R  V  G   1400

     ---------|---------|---------|---------|---------|---------|
4201 GAGGAGGAGATCCTGATCTACATCAATGACCATGAGGACAAAAACGAAGAGGCATTTTGC 4260
1401 E  E  E  I  L  I  Y  I  N  D  H  E  D  K  N  E  E  A  F  C   1420

     ---------|---------|-
4261 GTGAAGGTCATCTACCAGTGA 4281
1421 V  K  V  I  Y  Q  *   1426

    GGGCTTGAGGGTGACGTCCTTCCTGCGGCACCCAGCTGGGGCCTGTCTGTGCCCCTCCTG
    CCCTGCAGGCTGTCCTCCCCGCCTCTCTGCAGCCTTTCACTTCAGTGCCCACCTGGCTGA
    CCTGTGCACTTGGCTGAGGAAGCAGAGACCGAGCGCTGGTCATTTTGTAGTACCTGCATC
    CAGCTTAGCTGCTGCTGACACCCAGCAGGCCTGGGTTCCGTGAGCGCGAACTCCGTGGTG
    GTGGGTCTGGCTCTGGTGCTGCCATCTACGCATGTGGGACCCTCGTTATCGCTGTTGCTC
    AAAATGTATTTTATGAATCATCCTAAATGAGAAAATTATGTTTTTCTTACTGGATTTTGT
    ACAAACATAATCTATTATTTGCTATGCAATATTTTATGCTGGTATTATATCTGTTTTTTA
    AATTGTTGAACAAAATACTAAACTTTT
```

Exon 30

Figure 5



```
human  MNDWHRIFTQ NVLVPPHPQR ARQPWKESTA FQCVLKWLDG PVIRQ  45
mouse  MGDWHRAFTQ NTLVPPHPQR ARQLGKESTA FQCILKWLDG PLIKQ  45
CEleg  .......... .......... .......... .......... .....   0

human  GVLEVLSEVE CHLRVSFFDV TYRHFFGRTW KTTVKPTKRP ...PS  87
mouse  GILDMLSELE CHLRVTLFDV TYKHFFGRTW KTTVKPTNQP SKQPP  90
CEleg  .......... ....MSVNDW YSLFLANRPV EMKRNVSRGT KALCY  31

human  RIVFNEP... ..LYFHTSLN HPHIVAVVEV VAEGKKR... ....D 120
mouse  RITFNEP... ..LYFHTTLS HPSIVAVVEV VTEGRKR... ....D 123
CEleg  SMFISNLTSP QTLYFYSIIN SRDVLLILEF VEEGSDEING RTFEN  76

human  GSLQTLSCGF GILRIFSN.. QPDSPISASQ DKRLRLYHGT PRALL 163
mouse  GTLQLLSCGF GILRIFGN.. KPESPTSAAQ DKRLRLYHGT PRALL 166
CEleg  PKSTKITAPA TSVGWFSTHI EKKTPVEISN TKIFDIFGGT PKLLI 121

human  HPLLQDPAEQ NRHMTLIENC SLQYTLKPHP ALEPAFHLLP ENLLV 208
mouse  HPLLQDPIEQ NKFMRLMENC SLQYTLKPHP PLEPAFHLLP ENLLV 211
CEleg  F.......DK ETVLKPVGNV ECTYNIFEMP PI..FFQCLP EFCIV 157

                                 NLS                    ☐
human  SGLQQIPGLL PAHGESGDAL RKPRLQKPIT GHLDDLFFTL YPSLE 253
mouse  SGFQQIPGLL PPHGDTGDAL RKPRFQKPTT WHLDDLFFTL YPSLE 256
CEleg  CDKDIIPGII KDSSD.EWWL STPKEMPTIP AATDAIVIQF KNNVP 201

         E-rich
human  KFEEELLELH VQDHFQEGCG PLDGGALEIL ERRLRVGVHN GLGFV 298
mouse  KFEEELVQLL ISD..REGVG LLDSGTLEVL ERRLHVCVHN GLGFV 299
CEleg  ELEKQITHDI EKEWALKEGG TLKPKAI.IM DRKLRIGVHN GYTYV 245

                                        S-rich
human  QRPQVVVLVP EMDVALTRSA SFSRKVVSSS KTSSGSQALV LRS.. 341
mouse  QRPQVVVLVP EMDVALTRSA SFSRKISASS KNSSGNQALV LRS.. 342
CEleg  TEPFTVDLEI ISSNAGDTLR SRKKPIDFGK SSNWEEQLLF QAAGN 290

human  .RLRLPEMVG HPAFAMIFQL EYVFSSPAGV DGNAASVTSL SNLAC 385
mouse  .HLRLPEMVS HPAFAIMFQL EYVFNSPSGA DGGASSPTSI SSVAC 386
CEleg  PRLALRNLYA DPRMAIIFLL EYTFHREDNQ SLNQTILIGW AAWTP 335

human  MHMVRWAVWN PLLEADSGRV TLPLQGGIQP NPSHCLVYKV PSASM 430
mouse  MHMVRWAVWN PDLEVGPGKV TLPLQGGVQQ NPSRCLVYKV PSASM 431
CEleg  FS.......D GAFSGKEVET RVSFVGGPRP NPEGVLCYKN VLNQP 373

                                               P-rich
human  SSEEVKQVES GTLRFQFSLG SEEHLDAPTE PVSGPKVERR PSRKP 475
mouse  SSEEVKQVES GTIQFQFSLS S....DGPTE HANGPRVGRR SSRKM 472
CEleg  DSLKPLNEKL EIFVDFKFYE NGRSVHNTPT SRRAADSARV QTGRS 418

                 P-rich
human  PTSPSSPPAP VPRVLAAPQN SPVGPGLSIS QLAASPRSPT QHCLA 520
mouse  PASPS..... .......... .......... .......... ..... 477
CEleg  GDNGQSARSN RKSVKIETPR SPENSN..RF PALVDTGRSV SSVDE 461

human  RPTSQLPHGS QASPAQAQEF PLEAGISHLE ADLSQTSLVL ETSIA 565
mouse  .......... .......QES VLESRVSHLE ADLSQPASLQ GTPAV 505
CEleg  LRSINEDLNR FIEEPMEIPV QDVVVAKKPV EEPLPITSVY KIPFD 506

human  EQLQELPFTP LHAPIVVGTQ TRSSAGQPSR ASMVLLQSSG FPEIL 610
mouse  EHLQELPFTP LHAPIVVGAQ TRSSRSQLSR AAMVLLQSSG FPEIL 550
CEleg  ELKPINFP.. .......... .........R SAHSMFARQN FTQSIK 530
```



```
human  DANKQPAEAV SATEPVTFNP QKEESDCLQS NEMVLQFLAF SRVAQ  655
mouse  DASQQPVEAV NPIDPVRFNP QKEESDCLRG NEIVLQFLAF SRAAQ  595
CEleg  DRNGSPPNTE DVTLKTIIDM KREQLDRLIT SHVYFQFIAF KQLAA  575

human  DCRGTSWPKT VYFTFQFYRF PPATTPRLQL VQLDEAGQPS SGALT  700
mouse  DCPGTPWPQT VYFTFQFYRF PPETTPRLQL VKLDGTGKSG SGSLS  640
CEleg  P..DARMIKK LFFTIGFYRF PDITTESMLL TSMEK.GEPT .....  612

human  HILVPVSRDG TFDA.GSPGF QLRYMVGPGF LKPGERRCFA RYLAV  744
mouse  HILVPINKDG SFDA.GSPGL QLRYMVDPGF LKPGEQRWFA HYLAA  684
CEleg  .LLTRLDKNG NSDVIASPGF IAKYIIEGEE SKAD....FL DFMAS  652

human  QTLQIDVWDG DSLLLIGSAA VQMKHLLRQG RPAVQASHEL EVVAT  789
mouse  QTLQVDVWDG DSLLLIGSAG VQMKHLLRQG RPAVQVSHEL EVVAT  729
CEleg  GHATIDVWDS DSLIHLGSTI VPIKNLYRRG REAVQLFIQC PVVDT  697

human  EYEQDNMVVS GDMLGFGRVK PIGVHSVVKG RLHLTLANVG HPCEQ  834
mouse  EYEQEMMAVS GDVAGFGSVK PIGVHTVVKG RLHLTLANVG HACEP  774
CEleg  SLDTSSKA.. .GAFLYMRVA NIGFPSGNTY DL........ .....  726

human  KVRGCSTLPP SRSRVISNDG AS.RFSGGSL LTTGSSRRKH VVQAQ  878
mouse  RARGSNLLPP SRSRVISNDG AS.FFSGGSL LIPGGPKRKR VVQAQ  818
CEleg  .SSSSSSLTT TRSNVNSGQG TVVRRLTSSI RLNEEGPHSY RIHAK  770

human  KLADVDSELA AMLLTHARQG KGPQDVSRES DATRRRKLER MRSVR  923
mouse  RLADVDSELA AMLLTHTRAG QGPQAAGQEA DAVHKRKLER MRLVR  863
CEleg  PLPGNSGVGL DRFLTAQRL. ...DIQQRHE QLFNENSLDK IRQWN  811

human  LQEAGGDLGR RGTSVLAQQS VRTQHLRDLQ VIAAYRERTK AESIA  968
mouse  LQEAGGDSDS RRISLLAQHS VRAQHSRDLQ VIDAYRERTK AESIA  908
CEleg  DLKEGFNFSD NKE..TAQKF IFEE...ELA AYKKLRYESK PAKLL  851

human  SLLSLAITTE HTLHATLGVA EFFEFVLKNP HNTQHTVTVE IDNPE 1013
mouse  GVLSQAITTH HTLYATLGTA EFFEFALKNP HNTQHTVAIE IDSPE  953
CEleg  EAVFKGITSC HQINPSFGEK VFFEFPLENY NSEPINCTIE FDDEA  896

human  LSVIVDSQEW RDFKGAAGLH TPVEEDMFHL RGSLAPQLYL RPHET 1058
mouse  LSIILDSQEW RYFKEATGLH TPLEEDMFHL RGSLAPQLYL RPRET  998
CEleg  LKPVFDAEEW .......... .......... .....KFYKT VNKVT  916

human  AHVPFKFQSF SAGQLAMVQA SPGLSNEKGM DAVSPWKSSA VPTKH 1103
mouse  AHIPLKFQSF SVGPLAPTQA PAEVITEKDA ESGPLWKCSA MPTKH 1043
CEleg  TPSEKQMMRQ TTDRIEICLQ PGDVLFIPFI YDAFFFPNDA FNMYS  961

human  AKVLFRA.SG GKPIAVLCLT VELQPHVVDQ VFRFYHPELS FLKKA 1147
mouse  AKVLFRV.ET GQLIAVLCLT VEPQPHVVDQ VFRFYHPELT FLKKA 1087
CEleg  TKVVFRRWDT KEPTATLDLH VHRRNFLLQH SVTFICETSG NWEKQ 1006

human  IRLPPWHTFP GAPVGMLGED PPVHVRCSDP NVICETQNVG PGEPR 1192
mouse  IRLPPWHTLP GAPVGMPGED PPVHVRCSDP NVICEAQNVG PGEPR 1132
CEleg  LVLPP..... .....MARDR RVLSCRCSDP SVRLTVRNAT LQQI. 1040

human  DIFLKVASGP SPEIKDFFVI IYSDRWLATP TQTWQVYLHS LQRVD 1237
mouse  DVFLKVASGP SPEIKDFFVV IYADRWLAVP VQTWQVCLHS LQRVD 1177
CEleg  .VGFTTYSGE TNDRKTELLL MYSDHWQTRL MATWKSTELP FFNVD 1084
```

```
human  VSCVAGQLTR LSLVLRGTQT VRKVRAFTSH PQELKTDPKG VFVLP 1282
mouse  VSCVAGQLTR LSLVLRGTQT VRKVRAFTSH PQELKTDPAG VFVLP 1222
CEleg  VRSIVGQTTR LHLLVHRRSE HDGVPDDLLK VYTASGCMKV VDSVL 1129

human  PRGVQDLHVG VRPLRAGSRF VHLNLVDVDC HQLVASWLVC LCCRQ 1327
mouse  PHGVQDLHVG VRPRRAGSRF VHLNLVDIDY HQLVASWLVC LSCRQ 1267
CEleg  TERTPTATID FTPNFIGTKK LVVSVVNTNT LKLERGFLVY GKSEA 1174

human  PLISKAFEIM LAAGEGKGVN KRITYTNPYP SRRTFHLHSD HPELL 1372
mouse  PLISKAFEIT MAAGDEKGTN KRITYTNPYP SRRTYRLHSD RPELL 1312
CEleg  PRITQKFVIQ IPSSDEAIRK VC........ .......... ..... 1196

human  RFREDSFQVG GGETYTIGLQ FAPSQRVGEE EILIYINDHE DKNEE 1417
mouse  RFKEDSFQVA GGETYTIGLR FLPSGSAGQE EILIYINDHE DKNEE 1357
CEleg  .......... .......... .......... .......... ..... 1196

           ER
human  AFCVKVIYQ1426
mouse  TFCVKVLYQ1366
CEleg  .........1196
```

Figure 6

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC         Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20     Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60     Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120    Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160    Exon 5

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200    Exon 6

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240    Exon 7

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900
281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300    Exon 8

    ---------|---------|---------|---------|---------|---------|
```

```
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC  960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F   320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R   340     Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L   360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L   380
                                                                         Exon 10
     ---------|---------|---------|---------|---------|---------|
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A   400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C   420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S   440     Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E   460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S   480

     ---------|---------|---------|---------|---------|---------|     Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P   500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560     Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A   520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F   540
                                                                          Exon 14
     ---------|---------|---------|---------|---------|---------|
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L   560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I   580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L   600     Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V   620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S   640

     ---------|---------|---------|---------|---------|---------|
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T   660

                                                                          Exon 16
```

```
     ---------|---------|---------|---------|---------|---------|
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661 S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T   680

     ---------|---------|---------|---------|---------|---------|
2041 CCACGACTGCAGCTGGTCCAGCTGGATGAGGCCGGCCAGCCCAGCTCTGGCGCCCTGACC 2100
 681 P  R  L  Q  L  V  Q  L  D  E  A  G  Q  P  S  S  G  A  L  T   700

     ---------|---------|---------|---------|---------|---------|
2101 CACATCCTCGTGCCTGTGAGCAGAGATGGCACCTTTGATGCTGGGTCTCCTGGCTTCCAG 2160
 701 H  I  L  V  P  V  S  R  D  G  T  F  D  A  G  S  P  G  F  Q   720

     ---------|---------|---------|---------|---------|---------|
2161 CTGAGGTACATGGTGGGCCCTGGGTTCCTGAAGCCAGGTGAGCGGCGCTGCTTTGCCCGC 2220   Exon 17
 721 L  R  Y  M  V  G  P  G  F  L  K  P  G  E  R  R  C  F  A  R   740

     ---------|---------|---------|---------|---------|---------|
2221 TACCTGGCCGTGCAGACCCTGCAGATTGACGTCTGGGACGGAGACTCCCTGCTGCTCATC 2280
 741 Y  L  A  V  Q  T  L  Q  I  D  V  W  D  G  D  S  L  L  L  I   760

     ---------|---------|---------|---------|---------|--F3↓C2335T
2281 GGATCTGCTGCCGTCCAGATGAAGCATCTCCTCCGCCAAGGCCGGCCGGCTGTGTAG--- 2340
 761 G  S  A  A  V  Q  M  K  H  L  L  R  Q  G  R  P  A  V  X  -   780
                                                                        Exon 18
```

Figure 7.

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC        Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20     Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60     Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120    Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480    Exon 5
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600    Exon 6
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720    Exon 7
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900    Exon 8
281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300
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```
     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F  320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R  340    Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L  360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L  380
                                                                        Exon 10
     ---------|---------|---------|---------|---------|---------|
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A  400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C  420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S  440    Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E  460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S  480

     ---------|---------|---------|---------|---------|---------|      Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P  500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560    Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A  520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F  540

     ---------|---------|---------|---------|---------|---------|      Exon 14
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L  560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I  580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L  600    Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V  620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S  640

     ---------|---------|---------|---------|---------|---------|
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T  660

                                                                        Exon 16
```

```
          ---------|---------|---------|---------|---------|---------|
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661 S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T   680

          ---------|---------|---------|---------|---------|---------|
2041 CCACGACTGCAGCTGGTCCAGCTGGATGAGGCCGGCCAGCCCAGCTCTGGCGCCCTGACC 2100
 681 P  R  L  Q  L  V  Q  L  D  E  A  G  Q  P  S  S  G  A  L  T   700

          ---------|---------|---------|---------|---------|---------|
2101 CACATCCTCGTGCCTGTGAGCAGAGATGGCACCTTTGATGCTGGGTCTCCTGGCTTCCAG 2160
 701 H  I  L  V  P  V  S  R  D  G  T  F  D  A  G  S  P  G  F  Q   720

          ---------|---------|---------|---------|---------|---------|
2161 CTGAGGTACATGGTGGGCCCTGGGTTCCTGAAGCCAGGTGAGCGGCGCTGCTTTGCCCGC 2220   Exon 17
 721 L  R  Y  M  V  G  P  G  F  L  K  P  G  E  R  R  C  F  A  R   740

          ---------|---------|---------|------F24↓G2260A---|---------|
2221 TACCTGGCCGTGCAGACCCTGCAGATTGACGTCTGGGACAGAGACTCCCTGCTGCTCATC 2280
 741 Y  L  A  V  Q  T  L  Q  I  D  V  W  D  R  D  S  L  L  L  I   760

          ---------|---------|---------|---------|---------|---------|
2281 GGATCTGCTGCCGTCCAGATGAAGCATCTCCTCCGCCAAGGCCGGCCGGCTGTGCAGGCC 2340
 761 G  S  A  A  V  Q  M  K  H  L  L  R  Q  G  R  P  A  V  Q  A   780
                                                                       Exon 18
          ---------|---------|---------|---------|---------|---------|
2341 TCCCACGAGCTTGAGGTCGTGGCAACTGAATACGAGCAGGACAACATGGTGGTGAGTGGA 2400
 781 S  H  E  L  E  V  V  A  T  E  Y  E  Q  D  N  M  V  V  S  G   800

          ---------|---------|---------|---------|---------|---------|
2401 GACATGCTGGGGTTTGGCCGCGTCAAGCCCATCGGCGTCCACTCGGTGGTGAAGGGCCGG 2460
 801 D  M  L  G  F  G  R  V  K  P  I  G  V  H  S  V  V  K  G  R   820

          ---------|---------|---------|---------|---------|---------|
2461 CTGCACCTGACTTTGGCCAACGTGGGTCACCCGTGTGAACAGAAAGTGAGAGGTTGTAGC 2520
 821 L  H  L  T  L  A  N  V  G  H  P  C  E  Q  K  V  R  G  C  S   840
                                                                       Exon 19
          ---------|---------|---------|---------|---------|---------|
2521 ACATTGCCACCGTCCAGATCTCGGGTCATCTCAAACGATGGAGCCAGCCGCTTCTCTGGA 2580
 841 T  L  P  P  S  R  S  R  V  I  S  N  D  G  A  S  R  F  S  G   860

          ---------|---------|---------|---------|---------|---------|
2581 GGCAGCCTCCTCACGACTGGAAGCTCAAGGCGAAAACACGTGGTGCAAGCACAGAAGCTG 2640
 861 G  S  L  L  T  T  G  S  S  R  R  K  H  V  V  Q  A  Q  K  L   880

          ---------|---------|---------|---------|---------|---------|
2641 GCGGACGTGGACAGTGAGCTGGCTGCCATGCTACTGACCCATGCCCGGCAGGGCAAGGGG 2700   Exon 20
 881 A  D  V  D  S  E  L  A  A  M  L  L  T  H  A  R  Q  G  K  G   900

          ---------|---------|---------|---------|---------|---------|
2701 CCCCAGGACGTCAGCCGCGAGTCGGATGCCACCCGCAGGCGTAAGCTGGAGCGGATGAGG 2760
 901 P  Q  D  V  S  R  E  S  D  A  T  R  R  R  K  L  E  R  M  R   920

          ---------|---------|---------|---------|---------|---------|
2761 TCTGTGCGCCTGCAGGAGGCCGGGGGAGACTTGGGCCGGCGCGGGACGAGCGTGTTGGCG 2820
 921 S  V  R  L  Q  E  A  G  G  D  L  G  R  R  G  T  S  V  L  A   940

          ---------|---------|---------|---------|---------|---------|
2821 CAGCAGAGCGTCCGCACACAGCACTTGCGGGACCTACAGGTCATCGCCGCCTACCGGGAA 2880   Exon 21
 941 Q  Q  S  V  R  T  Q  H  L  R  D  L  Q  V  I  A  A  Y  R  E   960

          ---------|---------|---------|---------|---------|---------|
2881 CGCACGAAGGCCGAGAGCATCGCCAGCCTGCTGAGCCTGGCCATCACCACGGAGCACACG 2940
 961 R  T  K  A  E  S  I  A  S  L  L  S  L  A  I  T  T  E  H  T   980

          ---------|---------|---------|---------|---------|---------|
2941 CTCCACGCCACGCTGGGGGTCGCCGAGTTCTTTGAGTTTGTGCTTAAGAACCCCCACAAC 3000
 981 L  H  A  T  L  G  V  A  E  F  F  E  F  V  L  K  N  P  H  N   1000

          ---------|---------|---------|---------|---------|---------|
3001 ACACAGCACACGGTGACTGTGGAGATCGACAACCCCGAGCTCAGCGTCATCGTGGACAGT 3060
```

```
1001 T  Q  H  T  V  T  V  E  I  D  N  P  E  L  S  V  I  V  D  S   1020

     ---------|---------|---------|---------|---------|---------|
3061 CAGGAGTGGAGGGACTTCAAGGGTGCTGCTGGCCTGCACACACCGGTGGAGGAGGACATG 3120   Exon 22
1021 Q  E  W  R  D  F  K  G  A  A  G  L  H  T  P  V  E  E  D  M   1040

     ---------|---------|---------|---------|---------|---------|
3121 TTCCACCTGCGTGGCAGCCTGGCCCCCCAGCTCTACCTGCGCCCCCACGAGACCGCCCAC 3180
1041 F  H  L  R  G  S  L  A  P  Q  L  Y  L  R  P  H  E  T  A  H   1060

     ---------|---------|---------|---------|---------|---------|
3181 GTCCCCTTCAAGTTCCAGAGCTTCTCTGCAGGGCAGCTGGCCATGGTGCAGGCCTCTCCT 3240
1061 V  P  F  K  F  Q  S  F  S  A  G  Q  L  A  M  V  Q  A  S  P   1080

     ---------|---------|---------|---------|---------|---------|   Exon 23
3241 GGGTTGAGCAACGAGAAGGGCATGGACGCCGTGTCACCTTGGAAGTCCAGCGCAGTGCCC 3300
1081 G  L  S  N  E  K  G  M  D  A  V  S  P  W  K  S  S  A  V  P   1100

     ---------|---------|---------|---------|---------|---------|
3301 ACTAAACACGCCAAGGTCTTGTTCCGAGCGAGTGGTGGCAAGCCCATCGCCGTGCTCTGC 3360
1101 T  K  H  A  K  V  L  F  R  A  S  G  G  K  P  I  A  V  L  C   1120   Exon 24

     ---------|---------|---------|---------|---------|---------|
3361 CTGACTGTGGAGCTGCAGCCCCACGTGGTGGACCAGGTCTTCCGCTTCTATCACCCGGAG 3420
1121 L  T  V  E  L  Q  P  H  V  V  D  Q  V  F  R  F  Y  H  P  E   1140

     ---------|---------|---------|---------|---------|---------|
3421 CTCTCCTTCCTGAAGAAGGCCATCCGCCTGCCGCCCTGGCACACATTTCCAGGTGCTCCG 3480
1141 L  S  F  L  K  K  A  I  R  L  P  P  W  H  T  F  P  G  A  P   1160   Exon 25

     ---------|---------|---------|---------|---------|---------|
3481 GTGGGAATGCTTGGTGAGGACCCCCCAGTCCATGTTCGCTGCAGCGACCCGAACGTCATC 3540
1161 V  G  M  L  G  E  D  P  P  V  H  V  R  C  S  D  P  N  V  I   1180

     ---------|---------|---------|---------|---------|---------|
3541 TGTGAGACCCAGAATGTGGGCCCCGGGGAACCACGGGACATATTTCTGAAGGTGGCCAGT 3600
1181 C  E  T  Q  N  V  G  P  G  E  P  R  D  I  F  L  K  V  A  S   1200   Exon 26

     ---------|---------|---------|---------|---------|---------|
3601 GGTCCAAGCCCGGAGATCAAAGACTTCTTTGTCATCATTTACTCGGATCGCTGGCTGGCG 3660
1201 G  P  S  P  E  I  K  D  F  F  V  I  I  Y  S  D  R  W  L  A   1220

     ---------|---------|---------|---------|---------|---------|
3661 ACACCCACACAGACGTGGCAGGTCTACCTCCACTCCCTGCAGCGCGTGGATGTCTCCTGC 3720
1221 T  P  T  Q  T  W  Q  V  Y  L  H  S  L  Q  R  V  D  V  S  C   1240   Exon 27

     ---------|---------|---------|---------|---------|---------|
3721 GTCGCAGGCCAGCTGACCCGCCTGTCCCTTGTCCTTCGGGGGACACAGACAGTGAGGAAA 3780
1241 V  A  G  Q  L  T  R  L  S  L  V  L  R  G  T  Q  T  V  R  K   1260

     ---------|---------|---------|---------|---------|---------|
3781 GTGAGAGCTTTCACCTCTCATCCCCAGGAGCTGAAGACAGACCCCAAAGGTGTCTTCGTG 3840
1261 V  R  A  F  T  S  H  P  Q  E  L  K  T  D  P  K  G  V  F  V   1280

     ---------|---------|---------|---------|---------|---------|   Exon 28
3841 CTGCCGCCTCGTGGGGTGCAGGACCTGCATGTTGGCGTGAGGCCCCTTAGGGCCGGCAGC 3900
1281 L  P  P  R  G  V  Q  D  L  H  V  G  V  R  P  L  R  A  G  S   1300

     ---------|---------|---------|---------|---------|---------|
3901 CGCTTTGTCCATCTCAACCTGGTGGACGTGGATTGCCACCAGCTGGTGGCCTCCTGGCTC 3960
1301 R  F  V  H  L  N  L  V  D  V  D  C  H  Q  L  V  A  S  W  L   1320

     ---------|---------|---------|---------|---------|---------|
3961 GTGTGCCTCTGCTGCCGCCAGCCGCTCATCTCCAAGGCCTTTGAGATCATGTTGGCTGCG 4020
1321 V  C  L  C  C  R  Q  P  L  I  S  K  A  F  E  I  M  L  A  A   1340

     ---------|---------|---------|---------|---------|---------|
4021 GGCGAAGGGAAGGGTGTCAACAAGAGGATCACCTACACCAACCCCTACCCCTCCCGGAGG 4080   Exon 29
1341 G  E  G  K  G  V  N  K  R  I  T  Y  T  N  P  Y  P  S  R  R   1360

     ---------|---------|---------|---------|---------|---------|
```

```
4081 ACATTCCACCTGCACAGCGACCACCCGGAGCTGCTGCGGTTCAGAGAGGACTCCTTCCAG 4140
1361 T  F  H  L  H  S  D  H  P  E  L  L  R  F  R  E  D  S  F  Q   1380

     ----------|----------|----------|----------|----------|----------|
4141 GTCGGGGGTGGAGAGACCTACACCATCGGCTTGCAGTTTGCGCCTAGTCAGAGAGTGGGT 4200
1381 V  G  G  G  E  T  Y  T  I  G  L  Q  F  A  P  S  Q  R  V  G   1400

     ----------|----------|----------|----------|----------|----------|
4201 GAGGAGGAGATCCTGATCTACATCAATGACCATGAGGACAAAAACGAAGAGGCATTTTGC 4260
1401 E  E  E  I  L  I  Y  I  N  D  H  E  D  K  N  E  E  A  F  C   1420

     ----------|----------|-
4261 GTGAAGGTCATCTACCAGTGA 4281
1421 V  K  V  I  Y  Q  *   1426

     GGGCTTGAGGGTGACGTCCTTCCTGCGGCACCCAGCTGGGGCCTGTCTGTGCCCCTCCTG
     CCCTGCAGGCTGTCCTCCCCGCCTCTCTGCAGCCTTTCACTTCAGTGCCCACCTGGCTGA
     CCTGTGCACTTGGCTGAGGAAGCAGAGACCGAGCGCTGGTCATTTTGTAGTACCTGCATC
     CAGCTTAGCTGCTGCTGACACCCAGCAGGCCTGGGTTCCGTGAGCGCGAACTCCGTGGTG
     GTGGGTCTGGCTCTGGTGCTGCCATCTACGCATGTGGGACCCTCGTTATCGCTGTTGCTC
     AAAATGTATTTTATGAATCATCCTAAATGAGAAAATTATGTTTTTCTTACTGGATTTTGT
     ACAAACATAATCTATTATTTGCTATGCAATATTTTATGCTGGTATTATATCTGTTTTTTA
     AATTGTTGAACAAAATACTAAACTTTT
```

Exon 30

Figure 8

```
     GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC        Exon 1
     TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
     TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
     AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
     GGAAGCCCACGGGACCCTCAGGCGGGCAGG
     ---------|---------|---------|---------|---------|---------|
   1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
   1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20    Exon 2

     ---------|---------|---------|---------|---------|---------|
  61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
  21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

     ---------|---------|---------|---------|---------|---------|
 .21 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
  41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60    Exon 3

     ---------|---------|---------|---------|---------|---------|
 .81 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
  61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

     ---------|---------|---------|---------|---------|---------|
 .41 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
  81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

     ---------|---------|---------|---------|---------|---------|
 .01 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
 .01 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120   Exon 4

     ---------|---------|---------|---------|---------|---------|
 .61 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
 .21 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

     ---------|---------|---------|---------|---------|---------|
 .21 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480   Exon 5
 .41 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160

     ---------|---------|---------|---------|---------|---------|
 .81 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
 .61 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

     ---------|---------|---------|---------|---------|---------|
 .41 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600   Exon 6
 .81 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200

     ---------|---------|---------|---------|---------|---------|
 .01 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
 .01 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

     ---------|---------|---------|---------|---------|---------|
 .61 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
 .21 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240   Exon 7

     ---------|---------|---------|---------|---------|---------|
 .21 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
 .41 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

     ---------|---------|---------|---------|---------|---------|
 .81 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
 .61 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

     ---------|---------|---------|---------|---------|---------|
                                                                        Exon 8
```

```
 841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG  900
 281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300

     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC  960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F   320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R   340     Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L   360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L   380

     ---------|---------|---------|---------|---------|---------|     Exon 10
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A   400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C   420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S   440     Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E   460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S   480

     ---------|---------|---------|---------|---------|---------|     Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P   500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560     Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A   520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F   540
                                                                       Exon 14
     ---------|---------|---------|---------|---------|---------|
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L   560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I   580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L   600     Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V   620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S   640
```

```
     ---------|---------|---------|---------|---------|---------|
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T   660

     ---------|---------|---------|---------|---------|---------|    Exon 16
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661 S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T   680

     ---------|---------|---------|---------|---------|---------|
2041 CCACGACTGCAGCTGGTCCAGCTGGATGAGGCCGGCCAGCCCAGCTCTGGCGCCCTGACC 2100
 681 P  R  L  Q  L  V  Q  L  D  E  A  G  Q  P  S  S  G  A  L  T   700

     ---------|---------|---------|---------|---------|---------|
2101 CACATCCTCGTGCCTGTGAGCAGAGATGGCACCTTTGATGCTGGGTCTCCTGGCTTCCAG 2160
 701 H  I  L  V  P  V  S  R  D  G  T  F  D  A  G  S  P  G  F  Q   720

     ---------|---------|---------|---------|---------|---------|
2161 CTGAGGTACATGGTGGGCCCTGGGTTCCTGAAGCCAGGTGAGCGGCGCTGCTTTGCCCGC 2220
 721 L  R  Y  M  V  G  P  G  F  L  K  P  G  E  R  R  C  F  A  R   740    Exon 17

     ---------|---------|---------|---------|---------|---------|
2221 TACCTGGCCGTGCAGACCCTGCAGATTGACGTCTGGGACGGAGACTCCCTGCTGCTCATC 2280
 741 Y  L  A  V  Q  T  L  Q  I  D  V  W  D  G  D  S  L  L  L  I   760

     ---------|---------|---------|---------|---------|---------|
2281 GGATCTGCTGCCGTCCAGATGAAGCATCTCCTCCGCCAAGGCCGGCCGGCTGTGCAGGCC 2340
 761 G  S  A  A  V  Q  M  K  H  L  L  R  Q  G  R  P  A  V  Q  A   780

     ---------|---------|---------|---------|---------|---------|    Exon 18
2341 TCCCACGAGCTTGAGGTCGTGGCAACTGAATACGAGCAGGACAACATGGTGGTGAGTGGA 2400
 781 S  H  E  L  E  V  V  A  T  E  Y  E  Q  D  N  M  V  V  S  G   800

     ---------|---------|---------|---------|---------|---------|
2401 GACATGCTGGGGTTTGGCCGCGTCAAGCCCATCGGCGTCCACTCGGTGGTGAAGGGCCGG 2460
 801 D  M  L  G  F  G  R  V  K  P  I  G  V  H  S  V  V  K  G  R   820

     ---------|---------|---------|---------|---------|---------|
2461 CTGCACCTGACTTTGGCCAACGTGGGTCACCCGTGTGAACAGAAAGTGAGAGGTTGTAGC 2520
 821 L  H  L  T  L  A  N  V  G  H  P  C  E  Q  K  V  R  G  C  S   840

     ---------|---------|---------|---------|---------|---------|    Exon 19
2521 ACATTGCCACCGTCCAGATCTCGGGTCATCTCAAACGATGGAGCCAGCCGCTTCTCTGGA 2580
 841 T  L  P  P  S  R  S  R  V  I  S  N  D  G  A  S  R  F  S  G   860

     ---------|---------|---------|---------|---------|---------|
2581 GGCAGCCTCCTCACGACTGGAAGCTCAAGGCGAAAACACGTGGTGCAAGCACAGAAGCTG 2640
 861 G  S  L  L  T  T  G  S  S  R  R  K  H  V  V  Q  A  Q  K  L   880

     ---------|---------|---------|---------|---------|---------|
2641 GCGGACGTGGACAGTGAGCTGGCTGCCATGCTACTGACCCATGCCCGGCAGGGCAAGGGG 2700    Exon 20
 881 A  D  V  D  S  E  L  A  A  M  L  L  T  H  A  R  Q  G  K  G   900

     ---------|---------|---------|---------|---------|---------|
2701 CCCCAGGACGTCAGCCGCGAGTCGGATGCCACCCGCAGGCGTAAGCTGGAGCGGATGAGG 2760
 901 P  Q  D  V  S  R  E  S  D  A  T  R  R  R  K  L  E  R  M  R   920

     ---------|---------|---------|---------|---------|---------|
2761 TCTGTGCGCCTGCAGGAGGCCGGGGGAGACTTGGGCCGGCGCGGGACGAGCGTGTTGGCG 2820
 921 S  V  R  L  Q  E  A  G  G  D  L  G  R  R  G  T  S  V  L  A   940

     ---------|---------|---------|---------|---------|---------|
2821 CAGCAGAGCGTCCGCACACAGCACTTGCGGGACCTACAGGTCATCGCCGCCTACCGGGAA 2880    Exon 21
 941 Q  Q  S  V  R  T  Q  H  L  R  D  L  Q  V  I  A  A  Y  R  E   960

     ---------|---------|---------|---------|---------|---------|
2881 CGCACGAAGGCCGAGAGCATCGCCAGCCTGCTGAGCCTGGCCATCACCACGGAGCACACG 2940
 961 R  T  K  A  E  S  I  A  S  L  L  S  L  A  I  T  T  E  H  T   980

     ---------|---------|---------|---------|---------|---------|
2941 CTCCACGCCACGCTGGGGGTCGCCGAGTTCTTTGAGTTTGTGCTTAAGAACCCCCACAAC 3000
 981 L  H  A  T  L  G  V  A  E  F  F  E  F  V  L  K  N  P  H  N   1000
```

```
     ----------|----------|----------|----------|----------|----------|
3001 ACACAGCACACGGTGACTGTGGAGATCGACAACCCCGAGCTCAGCGTCATCGTGGACAGT 3060
1001 T  Q  H  T  V  T  V  E  I  D  N  P  E  L  S  V  I  V  D  S   1020

     ----------|----------|----------|----------|----------|----------|   Exon 22
3061 CAGGAGTGGAGGGACTTCAAGGGTGCTGCTGGCCTGCACACACCGGTGGAGGAGGACATG 3120
1021 Q  E  W  R  D  F  K  G  A  A  G  L  H  T  P  V  E  E  D  M   1040

     ----------|----------|----------|----------|----------|----------|
3121 TTCCACCTGCGTGGCAGCCTGGCCCCCCAGCTCTACCTGCGCCCCCACGAGACCGCCCAC 3180
1041 F  H  L  R  G  S  L  A  P  Q  L  Y  L  R  P  H  E  T  A  H   1060

     ----------|----------|----------|----------|----------|----------|
3181 GTCCCCTTCAAGTTCCAGAGCTTCTCTGCAGGGCAGCTGGCCATGGTGCAGGCCTCTCCT 3240
1061 V  P  F  K  F  Q  S  F  S  A  G  Q  L  A  M  V  Q  A  S  P   1080

     ----------|----------|---------F30↓3272delT--------|----------|------- Exon 23
3241 GGGTTGAGCAACGAGAAGGGCATGGACGCCGG-TCACCTTGGAAGTCCAGCGCAGTGCCC 3300
1081 G  L  S  N  E  K  G  M  D  A  G   H  L  G  S  P  A  Q  C  P    1100

     ----------|----------|----------|----------|----------|----------|
3301 ACTAAACACGCCAAGGTCTTGTTCCGAGCGAGTGGTGGCAAGCCCATCGCCGTGCTCTGC 3360
1101  L  N  T  P  R  S  C  S  E  R  V  V  A  S  P  S  P  C  S  A 1120   Exon 24

     ----------|----------|----------|----------|----------|----------|
3361 CTGA
1121  X
```

Figure 9

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC        Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20      Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
 21 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60      Exon 3

    ---------|---------|---------|---------|---------|---------|
 81 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
 41 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
 01 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
 01 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120     Exon 4

    ---------|---------|---------|---------|---------|---------|
 61 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
 21 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
 21 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480     Exon 5
 41 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160

    ---------|---------|---------|---------|---------|---------|
 81 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
 61 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600     Exon 6
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
561 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720     Exon 7
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900     Exon 8
```

```
 281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300

     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F   320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R   340    Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L   360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L   380

     ---------|---------|---------|---------|---------|---------|          Exon 10
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A   400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C   420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S   440    Exon 11

     ---------|F32↓↓TC1334-1335AA
1321 GGTACACTCCGGTAA
 441 G  T  L  R  Y
```

Figure 10

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC         Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20    Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60    Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120   Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480   Exon 5
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600   Exon 6
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240   Exon 7

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
                                                                        Exon 8
```

```
 841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900
 281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R  300

     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F  320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R  340   Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L  360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L  380
                                                                      Exon 10
     ---------|---------|---------|---------|---------|---------|
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S. N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A  400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C  420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S  440   Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E  460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S  480

     ---------|---------|---------|---------|---------|---------|     Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P  500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560   Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A  520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F  540   Exon 14
     ---------|---------|---------|---------|---------|---------|
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L  560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I  580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L  600
                                                                      Exon 15
     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V  620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S  640
```

```
     ----------|----------|----------|----------|--------F60↓C1972T-|
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCTGA
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  X
```

Exon 16

Figure 11

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG        Exon 1
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20     Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60     Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120    Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160    Exon 5

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200    Exon 6

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240    Exon 7

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280
```

```
     ---------|---------|---------|---------|---------|---------|
 841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900    Exon 8
 281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300

     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F   320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R   340    Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L   360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L   380

     ---------|---------|---------|---------|---------|---------|    Exon 10
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A   400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C   420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S   440    Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E   460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S   480

     ---------|---------|---------|---------|---------|---------|    Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P   500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560    Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A   520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F   540

     ---------|---------|---------|---------|---------|---------|    Exon 14
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L   560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I   580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L   600    Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V   620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S   640
```

```
     ----------|----------|----------|----------|----------|----------|
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T   660

     ----------|----------|----------|----------|----------|----------|
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661 S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T   680

   F461↓C2044T
2041 CCATGA
 681 P  *
```

Exon 16

Figure 12

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC       Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA  60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20   Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60   Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120  Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480  Exon 5
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600  Exon 6
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240  Exon 7

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900  Exon 8
```

```
 281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300

     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F   320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R   340     Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L   360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L   380

     ---------|---------|---------|---------|---------|---------|     Exon 10
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A   400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C   420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S   440     Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E   460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S   480

     ---------|---------|---------|---------|---------|---------|
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500     Exon 12
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P   500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560     Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A   520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F   540
                                                                        Exon 14
     ---------|---------|---------|---------|---------|---------|
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L   560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I   580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L   600     Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V   620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S   640

     ---------|---------|---------|---------|---------|---------|
```

```
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T   660    Exon 16

     ---------|---------|---------|---------|---------|---------|
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661 S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T   680

     ---------|---------|---------|---------|---------|---------|
2041 CCACGACTGCAGCTGGTCCAGCTGGATGAGGCCGGCCAGCCCAGCTCTGGCGCCCTGACC 2100
 681 P  R  L  Q  L  V  Q  L  D  E  A  G  Q  P  S  S  G  A  L  T   700

     ---------|---------|---------|---------|---------|---------|
2101 CACATCCTCGTGCCTGTGAGCAGAGATGGCACCTTTGATGCTGGGTCTCCTGGCTTCCAG 2160
 701 H  I  L  V  P  V  S  R  D  G  T  F  D  A  G  S  P  G  F  Q   720

     ---------|---------|---------|---------|---------|---------|
2161 CTGAGGTACATGGTGGGCCCTGGGTTCCTGAAGCCAGGTGAGCGGCGCTGCTTTGCCCGC 2220
 721 L  R  Y  M  V  G  P  G  F  L  K  P  G  E  R  R  C  F  A  R   740    Exon 17

     ---------|---------|---------|---------|---------|---------|
2221 TACCTGGCCGTGCAGACCCTGCAGATTGACGTCTGGGACGGAGACTCCCTGCTGCTCATC 2280
 741 Y  L  A  V  Q  T  L  Q  I  D  V  W  D  G  D  S  L  L  L  I   760

     ---------|---------|---------|---------|---------|---------|
2281 GGATCTGCTGCCGTCCAGATGAAGCATCTCCTCCGCCAAGGCCGGCCGGCTGTGCAGGCC 2340
 761 G  S  A  A  V  Q  M  K  H  L  L  R  Q  G  R  P  A  V  Q  A   780
                                                                          Exon 18
     ---------|---------|---------|---------|---------|---------|
2341 TCCCACGAGCTTGAGGTCGTGGCAACTGAATACGAGCAGGACAACATGGTGGTGAGTGGA 2400
 781 S  H  E  L  E  V  V  A  T  E  Y  E  Q  D  N  M  V  V  S  G   800

     ---------|---------|---------|---------|---------|---------|
2401 GACATGCTGGGGTTTGGCCGCGTCAAGCCCATCGGCGTCCACTCGGTGGTGAAGGGCCGG 2460
 801 D  M  L  G  F  G  R  V  K  P  I  G  V  H  S  V  V  K  G  R   820

     ---------|---------|---------|---------|---------|---------|
2461 CTGCACCTGACTTTGGCCAACGTGGGTCACCCGTGTGAACAGAAAGTGAGAGGTTGTAGC 2520
 821 L  H  L  T  L  A  N  V  G  H  P  C  E  Q  K  V  R  G  C  S   840
                                                                          Exon 19
     ---------|-------F461↓C2542T-|---------|---------|---------|
2521 ACATTGCCACCGTCCAGATCTTGGGTCATCTCAAACGATGGAGCCAGCCGCTTCTCTGGA 2580
 841 T  L  P  P  S  R  S  W  V  I  S  N  D  G  A  S  R  F  S  G   860

     ---------|---------|---------|---------|---------|---------|
2581 GGCAGCCTCCTCACGACTGGAAGCTCAAGGCGAAAACACGTGGTGCAAGCACAGAAGCTG 2640
 861 G  S  L  L  T  T  G  S  S  R  R  K  H  V  V  Q  A  Q  K  L   880
                                                                          Exon 20
     ---------|---------|---------|---------|---------|---------|
2641 GCGGACGTGGACAGTGAGCTGGCTGCCATGCTACTGACCCATGCCCGGCAGGGCAAGGGG 2700
 881 A  D  V  D  S  E  L  A  A  M  L  L  T  H  A  R  Q  G  K  G   900

     ---------|---------|---------|---------|---------|---------|
2701 CCCCAGGACGTCAGCCGCGAGTCGGATGCCACCCGCAGGCGTAAGCTGGAGCGGATGAGG 2760
 901 P  Q  D  V  S  R  E  S  D  A  T  R  R  R  K  L  E  R  M  R   920

     ---------|---------|---------|---------|---------|---------|
2761 TCTGTGCGCCTGCAGGAGGCCGGGGGAGACTTGGGCCGGCGCGGGACGAGCGTGTTGGCG 2820
 921 S  V  R  L  Q  E  A  G  G  D  L  G  R  R  G  T  S  V  L  A   940

     ---------|---------|---------|---------|---------|---------|
2821 CAGCAGAGCGTCCGCACACAGCACTTGCGGGACCTACAGGTCATCGCCGCCTACCGGGAA 2880
 941 Q  Q  S  V  R  T  Q  H  L  R  D  L  Q  V  I  A  A  Y  R  E   960    Exon 21
     ---------|---------|---------|---------|---------|---------|
2881 CGCACGAAGGCCGAGAGCATCGCCAGCCTGCTGAGCCTGGCCATCACCACGGAGCACACG 2940
 961 R  T  K  A  E  S  I  A  S  L  L  S  L  A  I  T  T  E  H  T   980

     ---------|---------|---------|---------|---------|---------|
2941 CTCCACGCCACGCTGGGGGTCGCCGAGTTCTTTGAGTTTGTGCTTAAGAACCCCCACAAC 3000
 981 L  H  A  T  L  G  V  A  E  F  F  E  F  V  L  K  N  P  H  N   1000
```

```
     ---------|---------|---------|---------|---------|---------|
3001 ACACAGCACACGGTGACTGTGGAGATCGACAACCCCGAGCTCAGCGTCATCGTGGACAGT 3060
1001 T  Q  H  T  V  T  V  E  I  D  N  P  E  L  S  V  I  V  D  S   1020

     ---------|---------|---------|---------|---------|---------|    Exon 22
3061 CAGGAGTGGAGGGACTTCAAGGGTGCTGCTGGCCTGCACACACCGGTGGAGGAGGACATG 3120
1021 Q  E  W  R  D  F  K  G  A  A  G  L  H  T  P  V  E  E  D  M   1040

     ---------|---------|---------|---------|---------|---------|
3121 TTCCACCTGCGTGGCAGCCTGGCCCCCCAGCTCTACCTGCGCCCCCACGAGACCGCCCAC 3180
1041 F  H  L  R  G  S  L  A  P  Q  L  Y  L  R  P  H  E  T  A  H   1060

     ---------|---------|---------|---------|---------|---------|
3181 GTCCCCTTCAAGTTCCAGAGCTTCTCTGCAGGGCAGCTGGCCATGGTGCAGGCCTCTCCT 3240
1061 V  P  F  K  F  Q  S  F  S  A  G  Q  L  A  M  V  Q  A  S  P   1080

     ---------|---------|---------|---------|---------|---------|    Exon 23
3241 GGGTTGAGCAACGAGAAGGGCATGGACGCCGTGTCACCTTGGAAGTCCAGCGCAGTGCCC 3300
1081 G  L  S  N  E  K  G  M  D  A  V  S  P  W  K  S  S  A  V  P   1100

     ---------|---------|---------|---------|---------|---------|
3301 ACTAAACACGCCAAGGTCTTGTTCCGAGCGAGTGGTGGCAAGCCCATCGCCGTGCTCTGC 3360
1101 T  K  H  A  K  V  L  F  R  A  S  G  G  K  P  I  A  V  L  C   1120    Exon 24

     ---------|---------|---------|---------|---------|---------|
3361 CTGACTGTGGAGCTGCAGCCCCACGTGGTGGACCAGGTCTTCCGCTTCTATCACCCGGAG 3420
1121 L  T  V  E  L  Q  P  H  V  V  D  Q  V  F  R  F  Y  H  P  E   1140

     ---------|---------|---------|---------|---------|---------|
3421 CTCTCCTTCCTGAAGAAGGCCATCCGCCTGCCGCCCTGGCACACATTTCCAGGTGCTCCG 3480
1141 L  S  F  L  K  K  A  I  R  L  P  P  W  H  T  F  P  G  A  P   1160    Exon 25

     ---------|---------|---------|---------|---------|---------|
3481 GTGGGAATGCTTGGTGAGGACCCCCCAGTCCATGTTCGCTGCAGCGACCCGAACGTCATC 3540
1161 V  G  M  L  G  E  D  P  P  V  H  V  R  C  S  D  P  N  V  I   1180

     ---------|---------|---------|---------|---------|---------|
3541 TGTGAGACCCAGAATGTGGGCCCCGGGGAACCACGGGACATATTTCTGAAGGTGGCCAGT 3600
1181 C  E  T  Q  N  V  G  P  G  E  P  R  D  I  F  L  K  V  A  S   1200    Exon 26

     ---------|---------|---------|---------|---------|---------|
3601 GGTCCAAGCCCGGAGATCAAAGACTTCTTTGTCATCATTTACTCGGATCGCTGGCTGGCG 3660
1201 G  P  S  P  E  I  K  D  F  F  V  I  I  Y  S  D  R  W  L  A   1220

     ---------|---------|---------|---------|---------|---------|
3661 ACACCCACACAGACGTGGCAGGTCTACCTCCACTCCCTGCAGCGCGTGGATGTCTCCTGC 3720
1221 T  P  T  Q  T  W  Q  V  Y  L  H  S  L  Q  R  V  D  V  S  C   1240    Exon 27

     ---------|---------|---------|---------|---------|---------|
3721 GTCGCAGGCCAGCTGACCCGCCTGTCCCTTGTCCTTCGGGGGACACAGACAGTGAGGAAA 3780
1241 V  A  G  Q  L  T  R  L  S  L  V  L  R  G  T  Q  T  V  R  K   1260

     ---------|---------|---------|---------|---------|---------|
3781 GTGAGAGCTTTCACCTCTCATCCCCAGGAGCTGAAGACAGACCCCAAAGGTGTCTTCGTG 3840
1261 V  R  A  F  T  S  H  P  Q  E  L  K  T  D  P  K  G  V  F  V   1280

     ---------|---------|---------|---------|---------|---------|    Exon 28
3841 CTGCCGCCTCGTGGGGTGCAGGACCTGCATGTTGGCGTGAGGCCCCTTAGGGCCGGCAGC 3900
1281 L  P  P  R  G  V  Q  D  L  H  V  G  V  R  P  L  R  A  G  S   1300

     ---------|---------|---------|---------|---------|---------|
3901 CGCTTTGTCCATCTCAACCTGGTGGACGTGGATTGCCACCAGCTGGTGGCCTCCTGGCTC 3960
1301 R  F  V  H  L  N  L  V  D  V  D  C  H  Q  L  V  A  S  W  L   1320

     ---------|---------|---------|---------|---------|---------|
3961 GTGTGCCTCTGCTGCCGCCAGCCGCTCATCTCCAAGGCCTTTGAGATCATGTTGGCTGCG 4020
1321 V  C  L  C  C  R  Q  P  L  I  S  K  A  F  E  I  M  L  A  A   1340

     ---------|---------|---------|---------|---------|---------|
4021 GGCGAAGGGAAGGGTGTCAACAAGAGGATCACCTACACCAACCCCTACCCCTCCCGGAGG 4080    Exon 29
1341 G  E  G  K  G  V  N  K  R  I  T  Y  T  N  P  Y  P  S  R  R   1360
```

```
     ---------|---------|---------|---------|---------|---------|
4081 ACATTCCACCTGCACAGCGACCACCCGGAGCTGCTGCGGTTCAGAGAGGACTCCTTCCAG 4140
1361 T  F  H  L  H  S  D  H  P  E  L  L  R  F  R  E  D  S  F  Q   1380

     ---------|---------|---------|---------|---------|---------|
4141 GTCGGGGGTGGAGAGACCTACACCATCGGCTTGCAGTTTGCGCCTAGTCAGAGAGTGGGT 4200
1381 V  G  G  G  E  T  Y  T  I  G  L  Q  F  A  P  S  Q  R  V  G   1400     Exon 30

     ---------|---------|---------|---------|---------|---------|
4201 GAGGAGGAGATCCTGATCTACATCAATGACCATGAGGACAAAAACGAAGAGGCATTTTGC 4260
1401 E  E  E  I  L  I  Y  I  N  D  H  E  D  K  N  E  E  A  F  C   1420

     ---------|---------|-
4261 GTGAAGGTCATCTACCAGTGA 4281
1421 V  K  V  I  Y  Q  *   1426

     GGGCTTGAGGGTGACGTCCTTCCTGCGGCACCCAGCTGGGGCCTGTCTGTGCCCCTCCTG
     CCCTGCAGGCTGTCCTCCCCGCCTCTCTGCAGCCTTTCACTTCAGTGCCCACCTGGCTGA
     CCTGTGCACTTGGCTGAGGAAGCAGAGACCGAGCGCTGGTCATTTTGTAGTACCTGCATC
     CAGCTTAGCTGCTGCTGACACCCAGCAGGCCTGGGTTCCGTGAGCGCGAACTCCGTGGTG
     GTGGGTCTGGCTCTGGTGCTGCCATCTACGCATGTGGGACCCTCGTTATCGCTGTTGCTC
     AAAATGTATTTTATGAATCATCCTAAATGAGAAAATTATGTTTTTCTTACTGGATTTTGT
     ACAAACATAATCTATTATTTGCTATGCAATATTTTATGCTGGTATTATATCTGTTTTTTA
     AATTGTTGAACAAAATACTAAACTTTT
```

Figure 13

```
    GACGCGAGGCGGGTTCTTGGACTGAGTGTGCGGCGCGGTGCGCCGCCTTCCGAGGCTCC        Exon 1
    TCCCGCGGGTGGCAGCGGACGGGGCGCGCCCCTCGGCCAGTCCTCGGTCCTCAGGCTTG
    TGGCTCCGTTGAGCACCGGCCGCCGGGCCTCTGGGTCCGTCGAGTGGAGACTCTCTGAA
    AAGCGTGGGCTCCGTGGCCTCCGGCGCGGCCGCGGCGGGTCGGTCTCCTAGATCATCCG
    GGAAGCCCACGGGACCCTCAGGCGGGCAGG
    ---------|---------|---------|---------|---------|---------|
  1 ATGAACGACTGGCACAGGATCTTCACCCAAAACGTGCTTGTCCCTCCCCACCCACAGAGA 60
  1 M  N  D  W  H  R  I  F  T  Q  N  V  L  V  P  P  H  P  Q  R   20    Exon 2

    ---------|---------|---------|---------|---------|---------|
 61 GCGCGCCAGCCTTGGAAGGAATCCACGGCATTCCAGTGTGTCCTCAAGTGGCTGGACGGA 120
 21 A  R  Q  P  W  K  E  S  T  A  F  Q  C  V  L  K  W  L  D  G   40

    ---------|---------|---------|---------|---------|---------|
121 CCGGTAATTAGGCAGGGCGTGCTGGAGGTACTGTCAGAGGTTGAATGCCATCTGCGAGTG 180
 41 P  V  I  R  Q  G  V  L  E  V  L  S  E  V  E  C  H  L  R  V   60    Exon 3

    ---------|---------|---------|---------|---------|---------|
181 TCTTTCTTTGATGTCACCTACCGGCACTTCTTTGGGAGGACGTGGAAAACCACAGTGAAG 240
 61 S  F  F  D  V  T  Y  R  H  F  F  G  R  T  W  K  T  T  V  K   80

    ---------|---------|---------|---------|---------|---------|
241 CCGACGAAGAGACCGCCGTCCAGGATCGTCTTTAATGAGCCCTTGTATTTCACACATCC 300
 81 P  T  K  R  P  P  S  R  I  V  F  N  E  P  L  Y  F  H  T  S   100

    ---------|---------|---------|---------|---------|---------|
301 CTAAACCACCCTCATATCGTGGCTGTGGTGGAAGTGGTCGCTGAGGGCAAGAAACGGGAT 360
101 L  N  H  P  H  I  V  A  V  V  E  V  V  A  E  G  K  K  R  D   120   Exon 4

    ---------|---------|---------|---------|---------|---------|
361 GGGAGCCTCCAGACATTGTCCTGTGGGTTTGGAATTCTTCGGATCTTCAGCAACCAGCCG 420
121 G  S  L  Q  T  L  S  C  G  F  G  I  L  R  I  F  S  N  Q  P   140

    ---------|---------|---------|---------|---------|---------|
421 GACTCTCCTATCTCTGCTTCCCAGGACAAAAGGTTGCGGCTGTACCATGGCACCCCCAGA 480
141 D  S  P  I  S  A  S  Q  D  K  R  L  R  L  Y  H  G  T  P  R   160   Exon 5

    ---------|---------|---------|---------|---------|---------|
481 GCCCTCCTGCACCCGCTTCTCCAGGACCCCGCAGAGCAAAACAGACACATGACCCTCATT 540
161 A  L  L  H  P  L  L  Q  D  P  A  E  Q  N  R  H  M  T  L  I   180

    ---------|---------|---------|---------|---------|---------|
541 GAGAACTGCAGCCTGCAGTACACGCTGAAGCCACACCCGGCCCTGGAGCCTGCGTTCCAC 600
181 E  N  C  S  L  Q  Y  T  L  K  P  H  P  A  L  E  P  A  F  H   200   Exon 6

    ---------|---------|---------|---------|---------|---------|
601 CTTCTTCCTGAGAACCTTCTGGTGTCTGGTCTGCAGCAGATACCTGGCCTGCTTCCAGCT 660
201 L  L  P  E  N  L  L  V  S  G  L  Q  Q  I  P  G  L  L  P  A   220

    ---------|---------|---------|---------|---------|---------|
661 CATGGAGAATCCGGCGACGCTCTCCGAAAGCCTCGCCTCCAGAAGCCCATCACGGGGCAC 720
221 H  G  E  S  G  D  A  L  R  K  P  R  L  Q  K  P  I  T  G  H   240   Exon 7

    ---------|---------|---------|---------|---------|---------|
721 TTGGATGACTTATTCTTCACCCTGTACCCCTCCCTGGAGAAGTTTGAGGAAGAGCTGCTG 780
241 L  D  D  L  F  F  T  L  Y  P  S  L  E  K  F  E  E  E  L  L   260

    ---------|---------|---------|---------|---------|---------|
781 GAGCTCCACGTCCAGGACCACTTCCAGGAGGGATGTGGCCCACTGGACGGTGGTGCCCTG 840
261 E  L  H  V  Q  D  H  F  Q  E  G  C  G  P  L  D  G  G  A  L   280

    ---------|---------|---------|---------|---------|---------|
841 GAGATCCTGGAGCGGCGCCTGCGTGTGGGCGTGCACAATGGTCTGGGCTTCGTGCAGAGG 900   Exon 8
```

```
 281 E  I  L  E  R  R  L  R  V  G  V  H  N  G  L  G  F  V  Q  R   300

     ---------|---------|---------|---------|---------|---------|
 901 CCGCAGGTCGTTGTACTGGTGCCTGAGATGGATGTGGCCTTGACGCGCTCAGCTAGCTTC 960
 301 P  Q  V  V  V  L  V  P  E  M  D  V  A  L  T  R  S  A  S  F   320

     ---------|---------|---------|---------|---------|---------|
 961 AGCAGGAAAGTGGTCTCCTCTTCCAAGACCAGCTCCGGGAGCCAAGCTCTGGTTTTGAGA 1020
 321 S  R  K  V  V  S  S  S  K  T  S  S  G  S  Q  A  L  V  L  R   340     Exon 9

     ---------|---------|---------|---------|---------|---------|
1021 AGCCGCCTCCGCCTCCCAGAGATGGTCGGCCACCCTGCATTTGCGGTCATCTTCCAGCTG 1080
 341 S  R  L  R  L  P  E  M  V  G  H  P  A  F  A  V  I  F  Q  L   360

     ---------|---------|---------|---------|---------|---------|
1081 GAGTACGTGTTCAGCAGCCCTGCAGGAGTGGACGGCAATGCAGCTTCGGTCACCTCTCTG 1140
 361 E  Y  V  F  S  S  P  A  G  V  D  G  N  A  A  S  V  T  S  L   380

     ---------|---------|---------|---------|---------|---------|     Exon 10
1141 TCCAACCTGGCATGCATGCACATGGTCCGCTGGGCTGTTTGGAACCCCTTGCTGGAAGCT 1200
 381 S  N  L  A  C  M  H  M  V  R  W  A  V  W  N  P  L  L  E  A   400

     ---------|---------|---------|---------|---------|---------|
1201 GATTCTGGAAGGGTGACCCTGCCTCTGCAGGGTGGGATCCAGCCCAACCCCTCGCACTGT 1260
 401 D  S  G  R  V  T  L  P  L  Q  G  G  I  Q  P  N  P  S  H  C   420

     ---------|---------|---------|---------|---------|---------|
1261 CTGGTCTACAAGGTACCCTCAGCCAGCATGAGCTCTGAAGAGGTGAAGCAGGTGGAGTCG 1320
 421 L  V  Y  K  V  P  S  A  S  M  S  S  E  E  V  K  Q  V  E  S   440     Exon 11

     ---------|---------|---------|---------|---------|---------|
1321 GGTACACTCCGGTTCCAGTTCTCGCTGGGCTCAGAAGAACACCTGGATGCACCCACGGAG 1380
 441 G  T  L  R  F  Q  F  S  L  G  S  E  E  H  L  D  A  P  T  E   460

     ---------|---------|---------|---------|---------|---------|
1381 CCTGTCAGTGGCCCCAAAGTGGAGCGGCGGCCTTCCAGGAAACCACCCACGTCCCCTTCG 1440
 461 P  V  S  G  P  K  V  E  R  R  P  S  R  K  P  P  T  S  P  S   480

     ---------|---------|---------|---------|---------|---------|     Exon 12
1441 AGCCCGCCAGCGCCAGTACCTCGAGTTCTCGCTGCCCCGCAGAACTCACCTGTGGGACCA 1500
 481 S  P  P  A  P  V  P  R  V  L  A  A  P  Q  N  S  P  V  G  P   500

     ---------|---------|---------|---------|---------|---------|
1501 GGGTTGTCAATTTCCCAGCTGGCGGCCTCCCCGCGGTCCCCGACTCAGCACTGCTTGGCC 1560     Exon 13
 501 G  L  S  I  S  Q  L  A  A  S  P  R  S  P  T  Q  H  C  L  A   520

     ---------|---------|---------|---------|---------|---------|
1561 AGGCCTACTTCACAGCTACCCCATGGCTCTCAGGCCTCCCCGGCCCAGGCACAGGAGTTC 1620
 521 R  P  T  S  Q  L  P  H  G  S  Q  A  S  P  A  Q  A  Q  E  F   540
                                                                       Exon 14
     ---------|---------|---------|---------|---------|---------|
1621 CCGTTGGAGGCCGGTATCTCCCACCTGGAAGCCGACCTGAGCCAGACCTCCCTGGTCCTG 1680
 541 P  L  E  A  G  I  S  H  L  E  A  D  L  S  Q  T  S  L  V  L   560

     ---------|---------|---------|---------|---------|---------|
1681 GAAACATCCATTGCCGAACAGTTACAGGAGCTGCCGTTCACGCCTTTGCATGCCCCTATT 1740
 561 E  T  S  I  A  E  Q  L  Q  E  L  P  F  T  P  L  H  A  P  I   580

     ---------|---------|---------|---------|---------|---------|
1741 GTTGTGGGAACCCAGACCAGGAGCTCTGCAGGGCAGCCCTCGAGAGCCTCCATGGTGCTC 1800
 581 V  V  G  T  Q  T  R  S  S  A  G  Q  P  S  R  A  S  M  V  L   600     Exon 15

     ---------|---------|---------|---------|---------|---------|
1801 CTGCAGTCCTCCGGCTTTCCCGAGATTCTGGATGCCAATAAACAGCCAGCCGAGGCTGTC 1860
 601 L  Q  S  S  G  F  P  E  I  L  D  A  N  K  Q  P  A  E  A  V   620

     ---------|---------|---------|---------|---------|---------|
1861 AGCGCTACAGAACCTGTGACGTTTAACCCTCAGAAGGAAGAATCAGATTGTCTACAAAGC 1920
 621 S  A  T  E  P  V  T  F  N  P  Q  K  E  E  S  D  C  L  Q  S   640

     ---------|---------|---------|---------|---------|---------|
```

```
1921 AACGAGATGGTGCTACAGTTTCTTGCCTTTAGCAGAGTGGCCCAGGACTGCCGAGGAACA 1980
 641 N  E  M  V  L  Q  F  L  A  F  S  R  V  A  Q  D  C  R  G  T    660      Exon 16

     ---------|---------|---------|---------|---------|---------|
1981 TCATGGCCAAAGACTGTGTATTTCACCTTCCAGTTCTACCGCTTCCCACCCGCAACGACG 2040
 661 S  W  P  K  T  V  Y  F  T  F  Q  F  Y  R  F  P  P  A  T  T    680

     ---------|---------|---------|---------|---------|---------|
2041 CCACGACTGCAGCTGGTCCAGCTGGATGAGGCCGGCCAGCCCAGCTCTGGCGCCCTGACC 2100
 681 P  R  L  Q  L  V  Q  L  D  E  A  G  Q  P  S  S  G  A  L  T    700

     ---------|---------|---------|---------|---------|---------|
2101 CACATCCTCGTGCCTGTGAGCAGAGATGGCACCTTTGATGCTGGGTCTCCTGGCTTCCAG 2160
 701 H  I  L  V  P  V  S  R  D  G  T  F  D  A  G  S  P  G  F  Q    720

     ---------|---------|---------|---------|---------|---------|
2161 CTGAGGTACATGGTGGGCCCTGGGTTCCTGAAGCCAGGTGAGCGGCGCTGCTTTGCCCGC 2220      Exon 17
 721 L  R  Y  M  V  G  P  G  F  L  K  P  G  E  R  R  C  F  A  R    740

     ---------|---------|---------|---------|---------|---------|
2221 TACCTGGCCGTGCAGACCCTGCAGATTGACGTCTGGGACGGAGACTCCCTGCTGCTCATC 2280
 741 Y  L  A  V  Q  T  L  Q  I  D  V  W  D  G  D  S  L  L  L  I    760

     ---------|---------|---------|---------|---------|---------|
2281 GGATCTGCTGCCGTCCAGATGAAGCATCTCCTCCGCCAAGGCCGGCCGGCTGTGCAGGCC 2340
 761 G  S  A  A  V  Q  M  K  H  L  L  R  Q  G  R  P  A  V  Q  A    780
                                                                           Exon 18
     ---------|---------|---F622↓G2368T-----|---------|---------|
2341 TCCCACGAGCTTGAGGTCGTGGCAACTTAA
 781 S  H  E  L  E  V  V  A  T  *
```

Figure 14

Nucleotide sequence:

```
   1 ggttgctccc ggttgctaag aagactatga acaagtcaga gaacctgctg tttgctggtt
  61 catcattagc atcacaagtc catgctgctg ccgttaatgg agataagggt gctctacaga
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 181 ttatgtattg cgtgttggct gacagattgg attgtgcaga tgctcttctg aaggcaggag
 241 cagatgtgaa taaaactgac catagccaga gaacagccct ccatcttgca gcccagaagg
 301 gaaattatcg tttcatgaaa ctcttactta cacgcagagc aaactggatg caaaaggatc
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 421 ttctgctgaa gtttatggca ccaggagaag tggatacaca ggataaaaac aagcaaacag
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 541 attctaacat tgggattcct gatgttgaag gcaagatccc acttcactgg gcagccaacc
 601 ataaagatcc aagtgctgtt cacacagtga gatgcattct ggatgctgct ccaacagagt
 661 ctttactgaa ctggcaagac tacgagggtc gaactcctct tcactttgca gttgctgatg
 721 ggaatgtgac cgtggttgat gtcttgacct catatgaaag ctgcaatata acgtcttatg
 781 ataacttatt tcgaacccca ctgcactggg cagctttatt aggccatgca cagattgtcc
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 901 ctttgcacta tgctgctcag agtaactttg ctgaaacggt taaagtgttt ttaaaacatc
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1081 tggctgacaa atatggaggt acagctttgc atgctgctgc tctttctggc catgtcagca
1141 ccgtgaagtt attactggaa aataatgctc aagtagatgc tactgatgtt atgaaacata
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1741 gaaaagcctt ccgagacagg aaaaatctcc tcatgaagca tgaacagttg agaaaagatg
1801 ctgctgccaa aaagcgagag gaagaaaaca aacgaaaaga ggcagaacag caaaaggaa
1861 ggcggagccc agattcctgc agaccccagg cccttccctg tctgcctagc acccaggatg
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2161 atggaagcag gcatccagga gttccctctg ttgagaagtc cagaggtgag acagctggcg
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2341 gccactggaa gcccagcagg cggcatgaca cagaacccaa ggccaaatgt gcccccccaga
2401 aaaggcgcac tcaagagctc agaggaggaa ggtgctctcc ggctggttct agccgccctg
2461 gcagtgcccg gggggaggcg gtccatgctg ggcagaatcc tccccaccat cgtacaccaa
2521 gaaacaaagt gacacaagcc aagctcacag gagggctcta ttcacatttg ccacagagca
2581 cagaggagtt gaggtcagga gctaggaggc tggagacatc taccctgtcc gaggactttc
2641 aggtatctaa ggagactgat ccagcacctg gtcccctctc tgggcagagt gtgaatattg
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2761 tgtttcgcaa aaagaacaag gcagcagcag tcatccagcg cgcctggcga agctaccagc
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2881 gatggaggca agagtctaca gcattgctcc tccaggtttg gaggaaggaa ctggaactaa
2941 aattccccca aaccactgca gtaagcaagg cccccaagag tccatccaag ggcacctcag
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3181 acctgcaatc agctactcag ccaaaaaaca aaacaaaacc ttgactgcct atggaggaag
3241 actgtgttcg ggggagctgg catagctagt gcagagttca gattttctgc tgataatctt
3301 ttacaccttg ggaaaacttt aatatccgta cctgaaggct gattcaccta aaaatgtgtt
3361 aactgaaaga aaatgtcaga atgtttcctt tctgctctta cacagcattg ttttgtcaat
3421 caacacagcc tgcactgaaa ggacctgcat agactatgtc tgtgcaaagt gcctgagtgt
3481 ctgctttcac ctcagtctgt acagttggaa atgagaattc ataattaaca gcaaaatcta
3541 aggaaaacta aaataaaa
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Amino acid sequence:

MNKSENLLFAGSSLASQVHAAAVNGDKGALQRLIVGNSALKDKE

DQFGRTPLMYCVLADRLDCADALLKAGADVNKTDHSQRTALHLAAQKGNYRFMKLLLT

RRANWMQKDLEEMTPLHLTTRHRSPKCLALLLKFMAPGEVDTQDKNKQTALHWSAYYN

NPEHVKLLIKHDSNIGIPDVEGKIPLHWAANHKDPSAVHTVRCILDAAPTESLLNWQD

YEGRTPLHFAVADGNVTVVDVLTSYESCNITSYDNLFRTPLHWAALLGHAQIVHLLLE

RNKSGTIPSDSQGATPLHYAAQSNFAETVKVFLKHPSVKDDSDLEGRTSFMWAAGKGS

DDVLRTMLSLKSDIDINMADKYGGTALHAAALSGHVSTVKLLLENNAQVDATDVMKHT

PLFRACEMGHKDVIQTLIKGGARVDLVDQDGHSLLHWAALGGNADVCQILIENKINPN

VQDYAGRTPLQCAAYGGYINCMAVLMENNADPNIQDKEGRTALHWSCNNGYLDAIKLL

LDFAAFPNQMENNEERYTPLDYALLGERHEVIQFMLEHGALSIAAIQDIAAFKIQAVY

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FVKQPSCIRVAGPDEKGEDSRRAGASLPPHDSHWKPSRRHDTEPKAKCAPQKRRTQEL

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SGARRLETSTLSEDFQVSKETDPAPGPLSGQSVNIDLLPVELRLQIIQRERRRKELFR

KKNKAAAVIQRAWRSYQLRKHLSHLRHMKQLGAGDVDRWRQESTALLLQVWRKELELK

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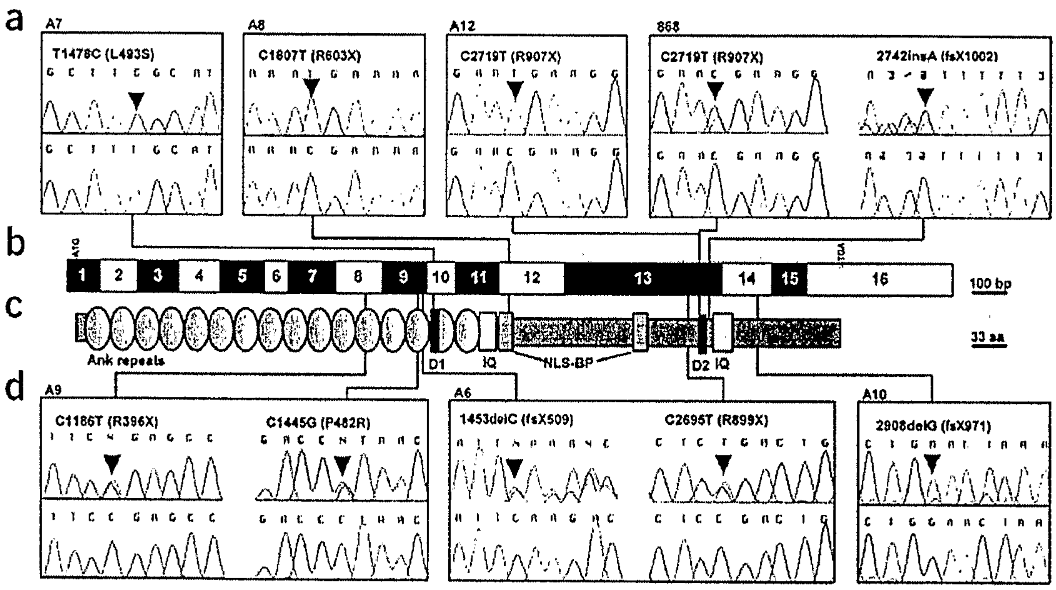
Figure 15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A7
T1478C (L493S)
A8
C1807T (R603X)
A12
C2719T (R907X)
868
C2719T (R907X)
2742insA (fsX1002)
b
ATG
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
TGA
100 bp
c
Ank repeats
D1
IQ
NLS-BP
D2
IQ
33 aa
d
A9
C1186T (R396X)
C1445G (P482R)
A6
1453delC (fsX509)
C2695T (R899X)
A10
2908delG (fsX971)

Figure 16

Nucleotide sequence:

C2695T

Amino Acid sequence:

R899X

Figure 17

Nucleotide sequence:

1453delC

Amino Acid sequence:

Q485fsX509

Figure 18

Nucleotide sequence:

C1807T

Amino Acid sequence:

R603X

Figure 19

Nucleotide sequence:

C1186T

Amino Acid sequence:

R396X

Figure 20

Nucleotide sequence:

C1445G

Amino Acid sequence:

P482R

Figure 21

Nucleotide sequence:

2908delG

Amino Acid sequence:

E970fsX971

Figure 22

Nucleotide sequence:

C2719T

Amino Acid sequence:

R907X

Figure 23

Nucleotide sequence:

C2719T

Amino Acid sequence:

R907X

Figure 24

Nucleotide sequence:

2747insA

Amino Acid sequence:

K916fsX1002

Figure 25

Nucleotide sequence:

T1478C

Amino Acid sequence:

L493S

Figure 26

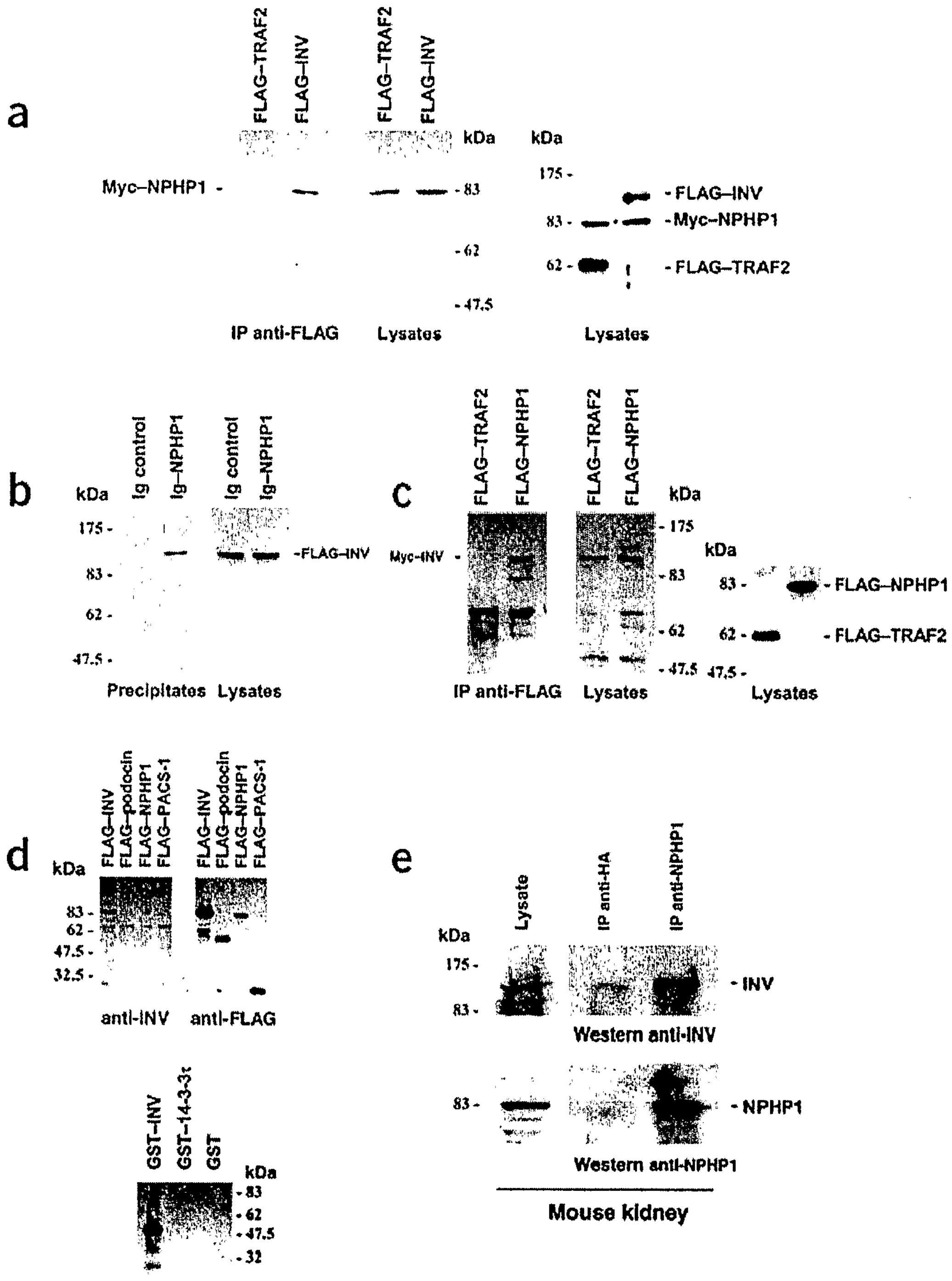

a
FLAG–TRAF2
FLAG–INV
FLAG–TRAF2
FLAG–INV
kDa
kDa
175
Myc–NPHP1
83
83
FLAG–INV
Myc–NPHP1
62
62
FLAG–TRAF2
47.5
IP anti-FLAG
Lysates
Lysates
b
kDa
Ig control
Ig–NPHP1
Ig control
Ig–NPHP1
175
FLAG–INV
83
62
47.5
Precipitates
Lysates
c
FLAG–TRAF2
FLAG–NPHP1
FLAG–TRAF2
FLAG–NPHP1
kDa
Myc–INV
175
83
kDa
83
FLAG–NPHP1
62
62
FLAG–TRAF2
47.5
47.5
IP anti-FLAG
Lysates
Lysates
d
kDa
FLAG–INV
FLAG–podocin
FLAG–NPHP1
FLAG–PACS-1
FLAG–INV
FLAG–podocin
FLAG–NPHP1
FLAG–PACS-1
83
62
47.5
32.5
anti-INV
anti-FLAG
GST–INV
GST–14-3-3ε
GST
kDa
83
62
47.5
32
e
kDa
Lysate
IP anti-HA
IP anti-NPHP1
175
INV
83
Western anti-INV
83
NPHP1
Western anti-NPHP1
Mouse kidney

Figure 27

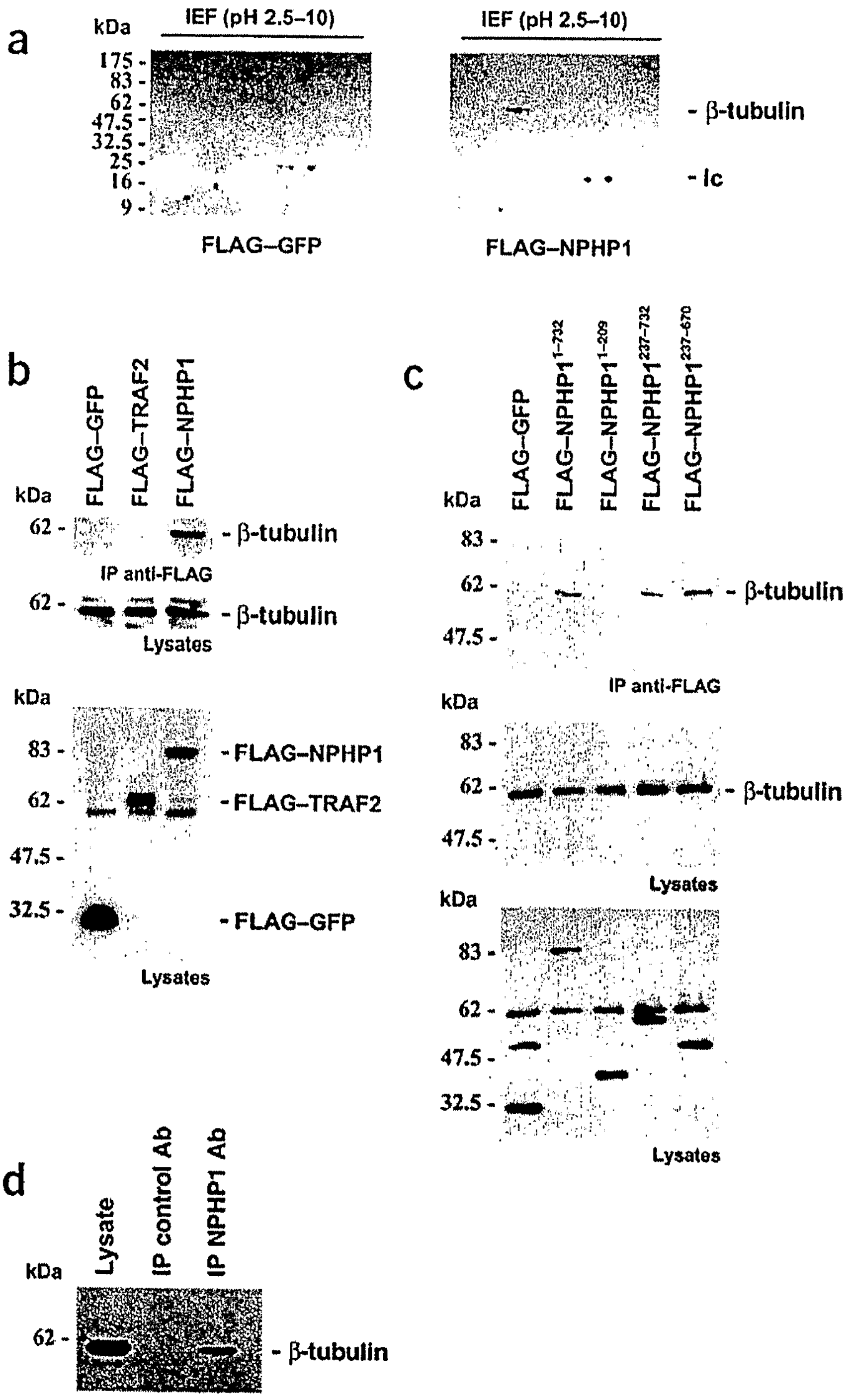

a
kDa
IEF (pH 2.5–10)
175
83
62
47.5
32.5
25
16
9
FLAG–GFP
IEF (pH 2.5–10)
β-tubulin
lc
FLAG–NPHP1
b
FLAG–GFP
FLAG–TRAF2
FLAG–NPHP1
kDa
62
β-tubulin
IP anti-FLAG
62
β-tubulin
Lysates
kDa
83
62
47.5
32.5
FLAG–NPHP1
FLAG–TRAF2
FLAG–GFP
Lysates
c
FLAG–GFP
FLAG–NPHP1^1–732
FLAG–NPHP1^1–209
FLAG–NPHP1^237–732
FLAG–NPHP1^237–670
kDa
83
62
47.5
β-tubulin
IP anti-FLAG
kDa
83
62
47.5
β-tubulin
Lysates
kDa
83
62
47.5
32.5
Lysates
d
Lysate
IP control Ab
IP NPHP1 Ab
kDa
62
β-tubulin

Figure 28

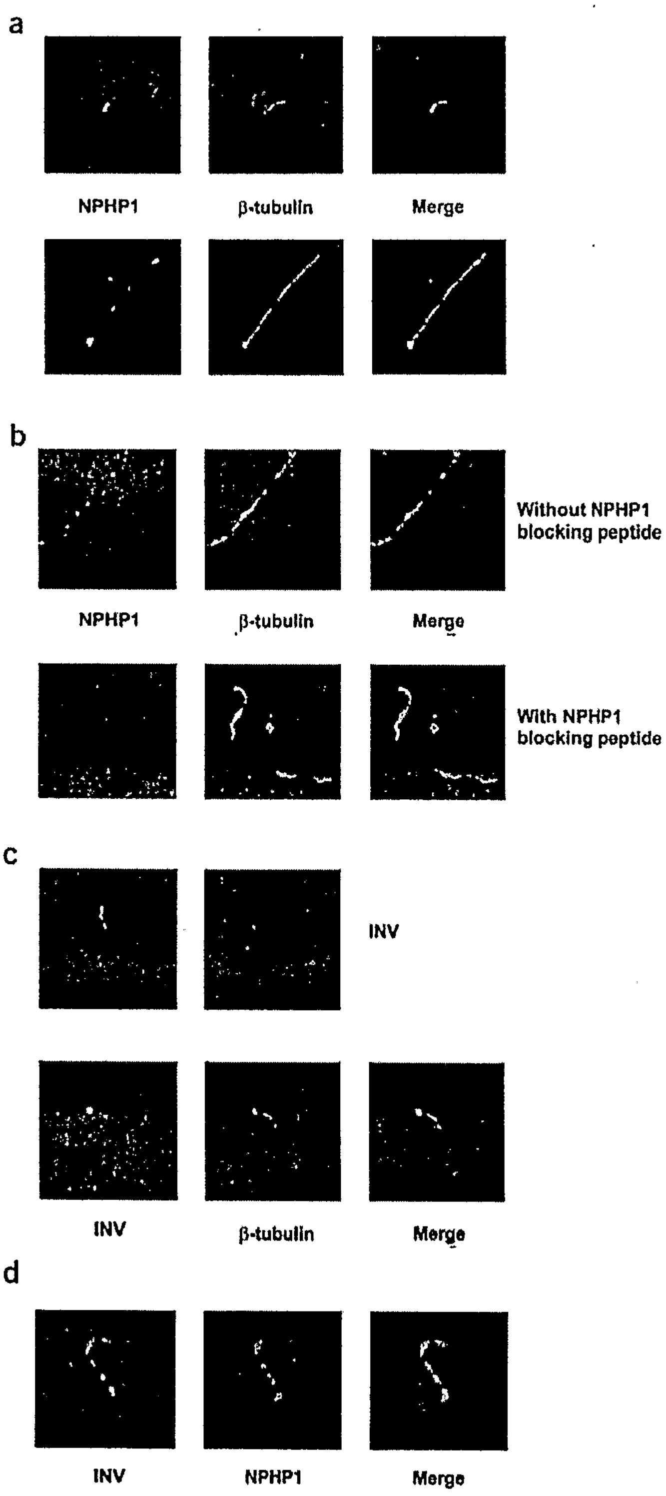
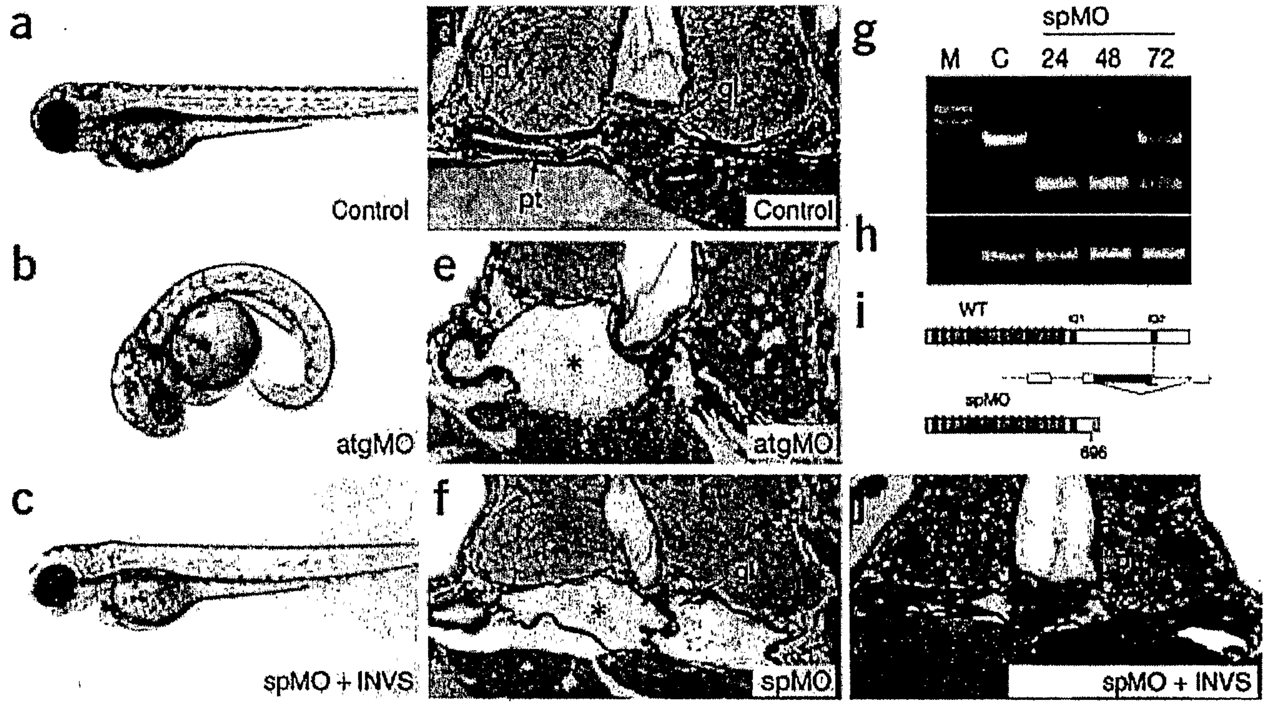

a
NPHP1
β-tubulin
Merge
b
Without NPHP1 blocking peptide
NPHP1
β-tubulin
Merge
With NPHP1 blocking peptide
c
INV
INV
β-tubulin
Merge
d
INV
NPHP1
Merge

Figure 29


a
Control
b
atgMO
c
spMO + INVS
d
gl
pt
Control
e
*
atgMO
f
*
gl
spMO
g
spMO
M
C
24
48
72
h
i
WT
spMO
696
j
gl
spMO + INVS