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(54) **Title**: COMPOSITIONS AND METHODS FOR TREATMENT OF KIDNEY DISEASES

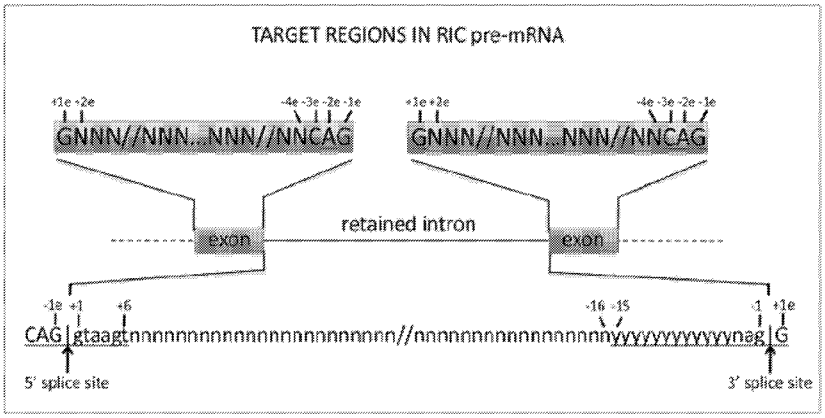

FIG. 1

(57) **Abstract**: Provided herein are methods and compositions for increasing the expression of a protein, and for treating a subject in need thereof, e.g., a subject with deficient protein expression or a subject having a kidney disease.

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COMPOSITIONS AND METHODS FOR TREATMENT OF KIDNEY DISEASES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/267,242, filed December 14, 2015, which application is incorporated herein by reference.

REFERENCE TO A SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on December 12, 2016, is named 47991-715_601_SL.txt and is 4,351,059 bytes in size. The aforementioned file was created on December 12, 2016, and is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Kidney disease is a debilitating and potentially fatal group of conditions associated with the damaging of the kidneys. The kidney is vital for many functions in the body including, but not limited to cleansing of the blood by removing waste and excess fluid, maintaining the balance of salt and minerals in the blood, and regulation of blood pressure. Incidence of kidney disease often results in the buildup of fluid and waste products, vomiting, weakness, poor sleep and shortness of breath, and ultimately could lead to death. While kidney transplantation can often prevent mortality, the odds of receiving a donor kidney is typically low.

[0004] While there are a large number of diseases and conditions associated with the kidney, a subset of kidney diseases have been shown to proceed via a deficiency in the expression of a gene, and in turn, a deficiency in the gene product. For example, *CTNS*, *PAX2*, *CYP24A1* and *PPARD*.

SUMMARY OF THE INVENTION

[0005] In one aspect, provided herein is a method of treating a kidney disease in a subject in need thereof by increasing the expression of a target protein or functional RNA by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site, and wherein the RIC pre-mRNA encodes the target protein or functional RNA, the method comprising contacting the cells of the subject with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein or functional RNA, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA, thereby increasing the

level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject.

[0006] In some embodiments, the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.

[0007] In one aspect, provided herein is a method of increasing expression of a target protein by cells having a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and wherein the RIC pre-mRNA encodes the target protein, the method comprising contacting the cells with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein, thereby increasing the level of mRNA encoding the target protein, and increasing the expression of the target protein in the cells, wherein the target protein is cystinosin, protein paired box gene 2 protein, protein cytochrome P450 family 24, subfamily A, polypeptide 1, or peroxisome proliferator activated receptor delta.

[0008] In some embodiments, the target protein is cystinosin, protein paired box gene 2 protein, protein cytochrome P450 family 24, subfamily A, polypeptide 1, or peroxisome proliferator activated receptor delta.

[0009] In some embodiments, the target protein or the functional RNA is a compensating protein or a compensating functional RNA that functionally augments or replaces a target protein or functional RNA that is deficient in amount or activity in the subject.

[0010] In some embodiments, the cells are in or from a subject having a condition caused by a deficient amount or activity of the target protein.

[0011] In some embodiments, the deficient amount of the target protein is caused by haploinsufficiency of the target protein, wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced, or a second allele encoding a nonfunctional target protein, and wherein the antisense oligomer binds to a targeted portion of a RIC pre-mRNA transcribed from the first allele.

[0012] In some embodiments, the subject has a condition caused by a disorder resulting from a deficiency in the amount or function of the target protein, wherein the subject has a first mutant allele from which the target protein is produced at a reduced level compared to production from a wild-type allele, the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or the target protein is not produced, and a second mutant allele from which the target protein is produced at a reduced level compared to production from a wild-type allele, the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or the target protein is not produced, and wherein when the subject has a first mutant allele a(iii), the second mutant

allele is b(i) or b(ii), and wherein when the subject has a second mutant allele b(iii), the first mutant allele is a(i) or a(ii), and wherein the RIC pre-mRNA is transcribed from either the first mutant allele that is a(i) or a(ii), and/or the second allele that is b(i)(ii) or b(ii).

[0013] In some embodiments, the target protein is produced in a form having reduced function compared to the equivalent wild-type protein.

[0014] In some embodiments, the target protein is produced in a form that is fully-functional compared to the equivalent wild-type protein.

[0015] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron.

[0016] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +69 relative to the 5' splice site of the retained intron to -79 relative to the 3' splice site of the retained intron.

[0017] In some embodiments, the target protein is cystinosin.

[0018] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[0019] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 8624-10068.

[0020] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748 or SEQ ID NO 10734.

[0021] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 8624-10068.

[0022] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 16 or SEQ ID NO 17.

[0023] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 3.

[0024] In some embodiments, the target protein is paired box gene 2 protein.

[0025] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[0026] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 6953-8623.

[0027] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10738 or SEQ ID NO 10740.

[0028] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 6953-8623.

[0029] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 10-15.

[0030] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 2.

[0031] In some embodiments, the target protein is protein cytochrome P450 family 24, subfamily A, polypeptide 1.

[0032] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[0033] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 9520-10733.

[0034] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748, SEQ ID NO 10734, SEQ ID NO 10746, SEQ ID NO 10745 or SEQ ID NO 10741.

[0035] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 9520-10733.

[0036] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 16-19.

[0037] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 4.

[0038] In some embodiments, the target protein is peroxisome proliferator activated receptor delta.

[0039] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[0040] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 20-6952.

[0041] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10736, SEQ ID NO 10747, SEQ ID NO 10739, SEQ ID NO 10743, SEQ ID NO 10742, SEQ ID NO 10737, SEQ ID NO 10735, or SEQ ID NO 10744.

[0042] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 20-6952.

[0043] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 5-9.

[0044] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 1.

[0045] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within: the region +6 to +100 relative to the 5' splice site of the retained intron; or the region -16 to -100 relative to the 3' splice site of the retained intron.

[0046] In some embodiments, the antisense oligomer targets a portion of the RIC pre-mRNA that is within the region about 100 nucleotides downstream of the 5' splice site of the at least one retained intron, to about 100 nucleotides upstream of the 3' splice site of the at least one retained intron

[0047] In some embodiments, the targeted portion of the RIC pre-mRNA is within: the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or the region +2e to -4e in the exon flanking the 3' splice site of the retained intron.

[0048] In some embodiments, the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating alternative splicing of pre-mRNA transcribed from a gene encoding the functional RNA or target protein.

[0049] In some embodiments, the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or the functional RNA.

[0050] In some embodiments, the RIC pre-mRNA was produced by partial splicing of a full-length pre-mRNA or partial splicing of a wild-type pre-mRNA.

[0051] In some embodiments, the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.

[0052] In some embodiments, the target protein produced is full-length protein, or wild-type protein.

[0053] In some embodiments, the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold,

about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell.

[0054] In some embodiments, the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of target protein produced by a control cell.

[0055] In some embodiments, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[0056] In some embodiments, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[0057] In some embodiments, the antisense oligomer comprises at least one modified sugar moiety.

[0058] In some embodiments, each sugar moiety is a modified sugar moiety.

[0059] In some embodiments, the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[0060] In some embodiments, the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the RIC pre-mRNA encoding the protein.

[0061] In some embodiments, the cell comprises a population of RIC pre-mRNAs transcribed from the gene encoding the target protein or functional RNA, wherein the population of RIC pre-mRNAs comprises two or more retained introns, and wherein the antisense oligomer binds to the most abundant retained intron in the population of RIC pre-mRNAs.

[0062] In some embodiments, the binding of the antisense oligomer to the most abundant retained intron induces splicing out of the two or more retained introns from the population of RIC pre-mRNAs to produce mRNA encoding the target protein or functional RNA.

[0063] In some embodiments, the cell comprises a population of RIC pre-mRNAs transcribed from the gene encoding the target protein or functional RNA, wherein the population of RIC pre-mRNAs comprises two or more retained introns, and wherein the antisense oligomer binds to the second most abundant retained intron in the population of RIC pre-mRNAs.

[0064] In some embodiments, the binding of the antisense oligomer to the second most abundant retained intron induces splicing out of the two or more retained introns from the population of RIC pre-mRNAs to produce mRNA encoding the target protein or functional RNA.

[0065] In some embodiments, the condition is a disease or disorder.

[0066] In some embodiments, the disease or disorder is a kidney disease.

[0067] In some embodiments, the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.

[0068] In some embodiments, the target protein and the RIC pre-mRNA are encoded by a gene, wherein the gene is CTNS, PAX2, CYP24A1 or PPARD.

[0069] In some embodiments, the method further comprises assessing protein expression.

[0070] In some embodiments, the subject is a human.

[0071] In some embodiments, the subject is a non-human animal.

[0072] In some embodiments, the subject is a fetus, an embryo, or a child.

[0073] In some embodiments, the cells are ex vivo.

[0074] In some embodiments, the antisense oligomer is administered by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject.

[0075] In some embodiments, the 9 nucleotides at -3e to -1e of the exon flanking the 5' splice site and +1 to +6 of the retained intron, are identical to the corresponding wild-type sequence.

[0076] In some embodiments, the 16 nucleotides at -15 to -1 of the retained intron and +1e of the exon flanking the 3' splice site are identical to the corresponding wild-type sequence.

[0077] In one aspect, provided herein is an antisense oligomer as used in a method described herein.

[0078] In one aspect, provided herein is an antisense oligomer comprising a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 20-10733.

[0079] In one aspect, provided herein is a pharmaceutical composition comprising an antisense oligomer described herein and an excipient.

[0080] In one aspect, provided herein is a method of treating a subject in need thereof by administering a pharmaceutical composition deribed herein by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[0081] In one aspect, provided herein is a composition comprising an antisense oligomer for use in a method of increasing expression of a target protein or a functional RNA by cells to treat a kidney disease in a subject in need thereof associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject, wherein the antisense oligomer enhances constitutive splicing of a retained intron-containing pre-mRNA (RIC pre-mRNA) encoding the target protein or the functional RNA, wherein the target protein is: the deficient protein; or a compensating protein which functionally augments or replaces the deficient protein or in the subject; and wherein the functional RNA is: the deficient RNA; or a compensating functional RNA which functionally augments or replaces the deficient functional RNA in the subject; wherein the RIC pre-mRNA comprises a retained intron, an exon flanking the 5' splice site and an exon flanking the 3' splice site, and wherein the retained intron is spliced from the RIC pre-mRNA encoding the target protein or the functional RNA, thereby increasing production or activity of the target protein or the functional RNA in the subject.

[0082] In some embodiments, the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.

[0083] In one aspect, provided herein is a composition comprising an antisense oligomer for use in a method of treating a condition associated with a target protein in a subject in need thereof, the method comprising the step of increasing expression of the target protein by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA) comprising a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and wherein the RIC pre-mRNA encodes the target protein, the method comprising contacting the cells with the antisense oligomer, whereby the retained intron is constitutively spliced from the RIC pre-mRNA transcripts encoding the target protein, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein, in the cells of the subject.

[0084] In some embodiments, the target protein is cystinosin, protein paired box gene 2 protein, protein cytochrome P450 family 24, subfamily A, polypeptide 1, or peroxisome proliferator activated receptor delta.

[0085] In some embodiments, the condition is a disease or disorder.

[0086] In some embodiments, the disease or disorder is a kidney disease.

[0087] In some embodiments, the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.

[0088] In some embodiments, the target protein and RIC pre-mRNA are encoded by a gene, wherein the gene is CTNS, PAX2, CYP24A1 or PPARD.

[0089] In some embodiments, the antisense oligomer targets a portion of the RIC pre-mRNA that is in the retained intron within the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron.

[0090] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +69 relative to the 5' splice site of the retained intron to -79 relative to the 3' splice site of the retained intron.

[0091] In some embodiments, the target protein is cystinosin.

[0092] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3'of the retained intron.

[0093] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 8624-10068.

[0094] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748 or SEQ ID NO 10734.

[0095] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 8624-10068.

[0096] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 16 or SEQ ID NO 17.

[0097] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 3.

[0098] In some embodiments, the target protein is paired box gene 2 protein.

[0099] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[00100] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 6953-8623.

[00101] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10738 or SEQ ID NO 10740.

[00102] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 6953-8623.

[00103] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 10-15.

[00104] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 2.

[00105] In some embodiments, the target protein is protein cytochrome P450 family 24, subfamily A, polypeptide 1.

[00106] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[00107] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 9520-10733.

[00108] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748, SEQ ID NO 10734, SEQ ID NO 10746, SEQ ID NO 10745 or SEQ ID NO 10741.

[00109] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 9520-10733.

[00110] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 16-19.

[00111] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 4.

[00112] In some embodiments, the target protein is peroxisome proliferator activated receptor delta.

[00113] In some embodiments, the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

[00114] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 20-6952.

[00115] In some embodiments, the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10736, SEQ ID NO 10747, SEQ ID NO 10739, SEQ ID NO 10743, SEQ ID NO 10742, SEQ ID NO 10737, SEQ ID NO 10735, or SEQ ID NO 10744.

[00116] In some embodiments, the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 20-6952.

[00117] In some embodiments, the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 5-9.

[00118] In some embodiments, the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 1.

[00119] In some embodiments, the antisense oligomer targets a portion of the RIC pre-mRNA that is in the retained intron within: the region +6 to +100 relative to the 5' splice site of the retained intron; or the region -16 to -100 relative to the 3' splice site of the retained intron.

[00120] In some embodiments, the antisense oligomer targets a portion of the RIC pre-mRNA that is within the region about 100 nucleotides downstream of the 5' splice site of the at least one retained intron, to about 100 nucleotides upstream of the 3' splice site of the at least one retained intron.

[00121] In some embodiments, the targeted portion of the RIC pre-mRNA is within: the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or the region +2e to -4e in the exon flanking the 3' splice site of the retained intron.

[00122] In some embodiments, the antisense oligomer does not increase the amount of target protein or functional RNA by modulating alternative splicing of the pre-mRNA transcribed from a gene encoding the target protein or functional RNA.

[00123] In some embodiments, the antisense oligomer does not increase the amount of the functional RNA or functional protein by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or functional RNA.

[00124] In some embodiments, the RIC pre-mRNA was produced by partial splicing from a full-length pre-mRNA or a wild-type pre-mRNA.

[00125] In some embodiments, the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.

[00126] In some embodiments, the target protein produced is full-length protein, or wild-type protein.

[00127] In some embodiments, the retained intron is a rate-limiting intron.

[00128] In some embodiments, said retained intron is the most abundant retained intron in said RIC pre-mRNA.

[00129] In some embodiments, the retained intron is the second most abundant retained intron in said RIC pre-mRNA.

[00130] In some embodiments, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00131] In some embodiments, said antisense oligomer is an antisense oligonucleotide.

[00132] In some embodiments, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[00133] In some embodiments, the antisense oligomer comprises at least one modified sugar moiety.

[00134] In some embodiments, each sugar moiety is a modified sugar moiety.

[00135] In some embodiments, the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[00136] In some embodiments, the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or is 100% complementary to the targeted portion of the RIC pre-mRNA encoding the protein.

[00137] In one aspect, provided herein is a pharmaceutical composition comprising an antisense oligomer described herein, and an excipient.

[00138] In one aspect, provided herein is a method of treating a subject in need thereof by administering a pharmaceutical composition described herein by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00139] In one aspect, provided herein is a pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of a deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript, wherein the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript comprises a retained intron, wherein the antisense oligomer induces splicing out of the retained intron from the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript; and a pharmaceutical acceptable excipient.

[00140] In some embodiments, the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript is a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript.

[00141] In some embodiments, the targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' spliced site of the retained intron.

[00142] In some embodiments, the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 1-4.

[00143] In some embodiments, the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 5-19.

[00144] In some embodiments, the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

[00145] In some embodiments, the antisense oligomer is an antisense oligonucleotide.

[00146] In some embodiments, the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

[00147] In some embodiments, the antisense oligomer comprises at least one modified sugar moiety.

[00148] In some embodiments, the antisense oligomer comprises from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

[00149] In some embodiments, the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or is 100% complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript.

[00150] In some embodiments, the targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript is within a sequence selected from SEQ ID NOs: 10034-10748.

[00151] In some embodiments, the antisense oligomer comprises a nucleotide sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 20-10733.

[00152] In some embodiments, the antisense oligomer comprises a nucleotide sequence selected from SEQ ID NOs: 20-10733.

[00153] In some embodiments, the pharmaceutical composition is formulated for intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

[00154] In one aspect, provided herein is a method of inducing processing of a deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript to facilitate removal of a retained intron to produce a fully processed CTNS, PAX2, CYP24A1 or PPARD mRNA transcript that encodes a functional form of a CTNS, PAX2, CYP24A1 or PPARD protein, the method comprising: contacting an antisense oligomer to a target cell of a subject; hybridizing the antisense oligomer to the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript, wherein the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript is capable of encoding the functional form of a CTNS, PAX2, CYP24A1 or PPARD protein and comprises at least one retained intron; removing the at least one retained intron from the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript to produce the fully processed CTNS, PAX2, CYP24A1 or PPARD mRNA transcript that encodes the functional form of CTNS, PAX2, CYP24A1 or PPARD protein; and translating the functional form of CTNS, PAX2, CYP24A1 or PPARD protein from the fully processed CTNS, PAX2, CYP24A1 or PPARD mRNA transcript.

[00155] In some embodiments, the retained intron is an entire retained intron.

[00156] In some embodiments, the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript is a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript.

[00157] In one aspect, provided herein is a method of treating a subject having a condition caused by a deficient amount or activity of CTNS, PAX2, CYP24A1 or PPARD protein comprising administering to the subject an antisense oligomer comprising a nucleotide sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 20-10733.

INCORPORATION BY REFERENCE

[00158] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[00159] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings.

[00160] **FIG. 1** depicts an schematic representation of an exemplary retained-intron-containing (RIC) pre-mRNA transcript. The 5' splice site consensus sequence is indicated with underlined letters (letters are nucleotides; upper case: exonic portion and lower case: intronic portion) from -3e to -1e and +1 to +6 (numbers labeled "e" are exonic and unlabeled numbers are intronic). The 3' splice site consensus sequence is indicated with underlined letters (letters are nucleotides; upper case: exonic portion and lower

case: intronic portion) from -15 to -1 and +1e (numbers labeled "e" are exonic and unlabeled numbers are intronic). Intronic target regions for ASO screening comprise nucleotides +6 relative to the 5' splice site of the retained intron (arrow at left) to -16 relative to the 3' splice site of the retained intron (arrow at right). In embodiments, intronic target regions for ASO screening comprise nucleotides +6 to +100 relative to the 5' splice site of the retained intron and -16 to -100 relative to the 3' splice site of the retained intron. Exonic target regions comprise nucleotides +2e to -4e in the exon flanking the 5' splice site of the retained intron and +2e to -4e in the exon flanking the 3' splice site of the retained intron. "n" or "N" denote any nucleotide, "y" denotes pyrimidine. The sequences shown represent consensus sequences for mammalian splice sites and individual introns and exons need not match the consensus sequences at every position.

[00161] FIG. 2A depicts an exemplary schematic representation of the Targeted Augmentation of Nuclear Gene Output (TANGO) approach. FIG. 2A shows a cell divided into nuclear and cytoplasmic compartments. In the nucleus, a pre-mRNA transcript of a target gene consisting of exons (rectangles) and introns (connecting lines) undergoes splicing to generate an mRNA, and this mRNA is exported to the cytoplasm and translated into target protein. For this target gene, the splicing of intron 1 is inefficient and a retained intron-containing (RIC) pre-mRNA accumulates primarily in the nucleus, and if exported to the cytoplasm, is degraded, leading to no target protein production.

[00162] FIG. 2B depicts an exemplary schematic representation of the Targeted Augmentation of Nuclear Gene Output (TANGO) approach. FIG. 2B shows an example of the same cell as in FIG. 2A divided into nuclear and cytoplasmic compartments. Treatment with an antisense oligomer (ASO) promotes the splicing of intron 1 and results in an increase in mRNA, which is in turn translated into higher levels of target protein.

[00163] FIG. 3A depicts a schematic of the ReSeq Genes for CTNS corresponding to NM_004937 and NM_001031681. The Percent Intron Retention (PIR) of the circled intron is shown.

[00164] FIG. 3B depicts a schematic of the ReSeq Genes for CTNS corresponding to NM_004937 and NM_001031681. The PIR of the circled intron is shown.

[00165] FIG. 3C depicts an exemplary graph showing the average (n=3) fold change in expression levels of CTNS mRNA without intron 10 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[00166] FIG. 4A depicts a schematic of the ReSeq Genes for CYP24A1 corresponding to NM_000782 and NM_001128915. The PIR of the circled intron is shown.

[00167] FIG. 4B depicts a schematic of the ReSeq Genes for CYP24A1 corresponding to NM_000782 and NM_001128915. The PIR of the circled intron is shown.

[00168] FIG. 4C depicts an exemplary graph showing the average (n=3) fold change in expression levels of CYP24A1 mRNA without intron 10 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[00169] FIG. 5 depicts a schematic of the ReSeq Genes for PAX2 corresponding to NM_001304569, NM_000278, NM_003990, NM_003988, NM_003987 and NM_003989.

[00170] FIG. 6A depicts a schematic of the RefSeq Genes for PPARD corresponding to PPARD: NM_006238, NM_177435, NM_001171818, NM_001171819 and NM_001171820. The Percent Intron Retention (PIR) of the circled intron is shown (PPARD intron 3, NM_006238).

[00171] FIG. 6B depicts an exemplary graph showing the average (n=3) fold change in expression levels of PPARD mRNA without intron 3 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[00172] FIG. 6C depicts a schematic of the RefSeq Genes for PPARD corresponding to PPARD: NM_006238, NM_177435, NM_001171818, NM_001171819 and NM_001171820. The Percent Intron Retention (PIR) of the circled intron is shown (PPARD intron 4, NM_006238).

[00173] FIG. 6D depicts a schematic of the RefSeq Genes for PPARD corresponding to PPARD: NM_006238, NM_177435, NM_001171818, NM_001171819 and NM_001171820. The Percent Intron Retention (PIR) of the circled intron is shown (PPARD intron 5, NM_006238).

[00174] FIG. 6E depicts a schematic of the RefSeq Genes for PPARD corresponding to PPARD: NM_006238, NM_177435, NM_001171818, NM_001171819 and NM_001171820. The Percent Intron Retention (PIR) of the circled intron is shown (PPARD intron 6, NM_006238).

[00175] FIG. 6F depicts an exemplary graph showing the average (n=3) fold change in expression levels of PPARD mRNA without intron 6 in ARPE-19 cells treated for 24 hrs with 80 nM of the indicated ASOs over mock treated cells. Data is normalized to RPL32 expression.

[00176] FIG. 6G depicts a schematic of the RefSeq Genes for PPARD corresponding to PPARD: NM_006238, NM_177435, NM_001171818, NM_001171819 and NM_001171820. The Percent Intron Retention (PIR) of the circled intron is shown (PPARD intron 7, NM_006238).

DETAILED DESCRIPTION OF THE INVENTION

[00177] Kidney failure is a debilitating condition that is estimated to affect more than 600,000 in the United States alone. In many cases, renal dialysis is able to prolong life, with an estimated 400,000 patients receiving renal dialysis according to a 2009 study. Still, the only hope for many afflicted with kidney failure is a kidney transplant, though the donor pool is only sufficient to treat a small portion of those in need. Therefore, the odds of receiving a transplant is low, and there are few treatments available to ameliorate the condition for those unable to receive a transplant. Therefore, there exists a need for compositions and methods for treating kidney diseases.

[00178] Individual introns in primary transcripts of protein-coding genes having more than one intron are spliced from the primary transcript with different efficiencies. In most cases only the fully spliced mRNA is exported through nuclear pores for subsequent translation in the cytoplasm. Unspliced and partially spliced transcripts are detectable in the nucleus. It is generally thought that nuclear accumulation of transcripts that are not fully spliced is a mechanism to prevent the accumulation of potentially deleterious mRNAs in the cytoplasm that may be translated to protein. For some genes, splicing of the least efficient intron is a rate-limiting post-transcriptional step in gene expression, prior to translation in the cytoplasm.

[00179] Substantial levels of partially-spliced transcripts of the gene, which encodes a protein that is deficient in a subset of kidney diseases, have been discovered in the nucleus of human cells. These pre-mRNA species comprise at least one retained intron. The present invention provides compositions and methods for upregulating splicing of one or more retained introns that are rate-limiting for the nuclear stages of gene expression to increase steady-state production of fully-spliced, mature mRNA, and thus, translated protein levels. These compositions and methods utilize antisense oligomers (ASOs) that promote constitutive splicing at an intron splice site of a retained-intron-containing pre-mRNA that accumulates in the nucleus. Thus, in embodiments, protein is increased using the methods of the invention to treat a condition caused by a protein deficiency.

[00180] Kidney diseases that can be treated by the invention described herein are diseases where a subject is deficient in a gene product, where deficiency in a gene product causes the kidney disease.

[0001] These CTNS, PAX2, CYP24A1 and PPARD pre-mRNA species comprise at least one retained intron. The present invention provides compositions and methods for upregulating splicing of one or more retained CTNS, PAX2, CYP24A1 or PPARD introns that are rate-limiting for the nuclear stages of gene expression to increase steady-state production of fully-spliced, mature mRNA, and thus, translated CTNS, PAX2, CYP24A1 or PPARD protein levels. These compositions and methods can utilize antisense oligomers (ASOs) that promote constitutive splicing at intron splice sites of a retained-intron-containing CTNS, PAX2, CYP24A1 or PPARD pre-mRNA (RIC pre-mRNA) that accumulates in the nucleus. Thus, in embodiments, CTNS, PAX2, CYP24A1 or PPARD protein can be increased using the methods of the invention to treat a condition caused by CTNS, PAX2, CYP24A1 or PPARD deficiency.

[0002] In other embodiments, the methods of the invention can be used to increase CTNS, PAX2, CYP24A1 or PPARD production to treat a condition in a subject in need thereof. In embodiments, the subject has a condition in which CTNS, PAX2, CYP24A1 or PPARD is not necessarily deficient relative to wild-type, but where an increase in CTNS, PAX2, CYP24A1 or PPARD mitigates the condition nonetheless. In embodiments, the condition can be caused by a CTNS, PAX2, CYP24A1 or PPARD haploinsufficiency.

Nephropathic Cystinosis/Late-Onset Cystinosis

[0003] In some embodiments, the invention described herein can be used to treat the autosomal recessive kidney disorder infantile nephropathic cystinosis. Infantile nephropathic cystinosis is characterized by the accumulation of cysteine in lysosomes. Clinical manifestations of infantile nephropathic cystinosis include low blood sugar and electrolytes, excessive excretion of proteins in the urine, slow body growth, weak bones, hypothyroidism, blindness, muscle weakness, pulmonary dysfunction and kidney failure. Typically infantile nephropathic cystinosis is diagnosed in infancy.

[0004] In some embodiments, the invention described herein can be used to treat the autosomal recessive kidney disorder late-onset cystinosis. Late onset cystinosis has a similar clinical manifestation as the infantile form, though the late onset form typically manifests in either late adolescence or early adulthood.

[0005] Deficiency in the protein cystinosin (CTNS) results in the clinical manifestations shown in both infantile nephropathic cystinosis and late-onset cystinosis. The *CTNS* gene, which is located at 17q13.2 and spans 12 exons, codes for the CTNS protein. Mutations in the *CTNS* gene resulting in deficient amounts of CTNS protein have been shown to be responsible for the progression of both infantile nephropathic cystinosis and late-onset cystinosis. In one study, a G169D missense mutation was discovered in patients with infantile nephropathic cystinosis. This missense mutation resulted in diminished levels of CTNS, which provided a positive link between deficiency in CTNS and the progression of infantile nephropathic cystinosis.

Focal Segmental Glomerulosclerosis 7/Papillorenal Syndrome

[0006] In some embodiments, the invention described herein can be used to treat the autosomal dominant kidney disorder focal segmental glomerulosclerosis 7 (FSGS7). FSGS7 is one of the leading causes of kidney failure in adults. FSGS7 is characterized by edema, hypoalbuminemia, hyperlipidemia and hypertension, which ultimately results in kidney failure.

[0007] In some embodiments, the invention described herein can be used to treat the autosomal dominant kidney disorder Papillorenal syndrome (PAPRS). PAPRS is characterized by hypoplastic kidneys, hypodysplasia, multicystic dysplastic kidney, oligomeganephronia, renal insufficiency and vesicoureteral reflux.

[0008] Deficiency in the protein paired box gene 2 (PAX2) results in the clinical manifestations shown in both FSGS7 and PAPRS. The *PAX2* gene, which is located at 10q24.31 and spans 12 exons, codes for the PAX2 protein. Mutations in the *PAX2* gene resulting in deficient amounts of PAX2 protein have been shown to be responsible for the progression of both FSGS7 and PAPRS. In one study, a G76S missense mutation was discovered in 5 generations of a family afflicted with PAPRS. This missense mutation resulted in diminished levels of PAX2 protein, which provided a positive link between deficiency in PAX2 protein and the progression of PAPRS.

Infantile Hypercalcemia

[0009] In some embodiments, the invention described herein can be used to treat the autosomal recessive kidney disease infantile hypercalcemia. Infantile hypercalcemia is characterized by elevated calcium levels in the blood, which can result in renal stones, bone pain, abdominal pain, nausea, vomiting, polyuria and psychiatric conditions such as depression, anxiety, cognitive dysfunction, insomnia and coma.

[0010] Deficiency in the protein cytochrome P450 family 24, subfamily A, polypeptide 1 (CYP24A1) results in the clinical manifestations shown in infantile hypercalcemia. The *CYP24A1* gene, which is located at 20q13.2 and spans 2 exons, codes for the CYP24A1 protein. Mutations in the *CYP24A1* gene resulting in deficient amounts of CYP24A1 protein have been shown to be responsible for the progression of infantile hypercalcemia. In one study, a number of children diagnosed with infantile hypercalcemia were examined. Children displaying an R396W or an E322K missense mutation were shown to have complete ablation of CYP24A1 activity, which children displaying an L409S mutation retained small, but measurable levels. This finding provides a positive link between diminished CYP24A1 protein and the clinical manifestations of infantile hypercalcemia.

Lipid Metabolism Dysfunction and Chronic Kidney Disease

[0011] In some embodiments, the invention described herein can be used to treat the lipid metabolism deficiency, chronic kidney disease (CKD), end-stage renal disease (ESRD) or cardiovascular disease (CVD) caused by deficiency in the protein peroxisome proliferator activated receptor delta (PPARD). Deficiency in the amount of PPARD protein, which is encoded by the *PPARD* gene located on chromosome 6 and spans 8 exons, has been shown to be correlated to increased lipid metabolism dysfunction.

Retained Intron Containing Pre-mRNA (RIC Pre-mRNA)

[0012] In embodiments, the methods of the present invention exploit the presence of retained-intron-containing pre-mRNA (RIC pre-mRNA) transcribed from a gene and encoding a protein that is found to be deficient in a disease described herein, in the cell nucleus. Splicing of the identified RIC pre-mRNA species to produce mature, fully-spliced, mRNA, is induced using ASOs that stimulate splicing out of the retained introns. The resulting mature mRNA can be exported to the cytoplasm and translated, thereby increasing the amount of protein in the patient's cells and alleviating symptoms of a disease or condition described herein. In embodiments, the methods of the present invention can exploit the presence of retained-intron-containing pre-mRNA (RIC pre-mRNA) transcribed from the *CTNS, PAX2, CYP24A1* or *PPARD* gene and encoding CTNS, PAX2, CYP24A1 or PPARD protein, in the cell nucleus. Splicing of CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA species to produce mature, fully-spliced, CTNS, PAX2, CYP24A1 or PPARD mRNA, can be induced using ASOs that stimulate splicing out of the retained introns. The resulting mature CTNS, PAX2, CYP24A1 or PPARD mRNA can be exported to the cytoplasm and translated, thereby increasing the amount of CTNS, PAX2, CYP24A1 or PPARD protein

in the patient's cells and alleviating symptoms of the CNS disease or conditions caused by deficiency in CTNS, PAX2, CYP24A1 or PPARD. This method, described further below, is known as Targeted Augmentation of Nuclear Gene Output (TANGO).

[0013] In embodiments, a retained intron is an intron that is identified as a retained intron based on a determination of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, or at least about 50%, retention. In embodiments, a retained intron is an intron that is identified as a retained intron based on a determination of about 5% to about 100%, about 5% to about 95%, about 5% to about 90%, about 5% to about 85%, about 5% to about 80%, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 10% to about 100%, about 10% to about 95%, about 10% to about 90%, about 10% to about 85%, about 10% to about 80%, about 10% to about 75%, about 10% to about 70%, about 10% to about 65%, about 10% to about 60%, about 10% to about 65%, about 10% to about 60%, about 10% to about 55%, about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 10% to about 20%, about 15% to about 100%, about 15% to about 95%, about 15% to about 90%, about 15% to about 85%, about 15% to about 80%, about 15% to about 75%, about 15% to about 70%, about 15% to about 65%, about 15% to about 60%, about 15% to about 65%, about 15% to about 60%, about 15% to about 55%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 100%, about 20% to about 95%, about 20% to about 90%, about 20% to about 85%, about 20% to about 80%, about 20% to about 75%, about 20% to about 70%, about 20% to about 65%, about 20% to about 60%, about 20% to about 65%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 45%, about 20% to about 40%, about 20% to about 35%, about 20% to about 30%, about 25% to about 100%, about 25% to about 95%, about 25% to about 90%, about 25% to about 85%, about 25% to about 80%, about 25% to about 75%, about 25% to about 70%, about 25% to about 65%, about 25% to about 60%, about 25% to about 65%, about 25% to about 60%, about 25% to about 55%, about 25% to about 50%, about 25% to about 45%, about 25% to about 40%, or about 25% to about 35%, retention. In embodiments, other ASOs useful for this purpose are identified, using, e.g., methods described herein.

[0014] In embodiments, the CTNS intron numbering corresponds to the mRNA sequence at NM_001031681 or NM_001031681. In embodiments, the targeted portion of the CTNS RIC pre-mRNA is in intron 9 and/or 10. In embodiments, the targeted portion of the CTNS RIC pre-mRNA is in intron 10. In embodiments, the percent retained intron can be 18%. In embodiments, the targeted portion of the

CTNS RIC pre-mRNA is in intron 9. In embodiments, the percent retained intron can be 10%. In embodiments, hybridization of an ASO to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of retained intron 9 and/or 10 and subsequently increases CTNS protein production. It is understood that the intron numbering may change in reference to a different CTNS isoform sequence. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_001031681 or NM_001031681. One of skill in the art also can determine the sequences of flanking exons in any CTNS isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_001031681 or NM_001031681.

[0015] In embodiments, the PAX2 intron numbering corresponds to the mRNA sequence at NM_001304569, NM_000278, NM_003990, NM_003988, NM_003987 or NM_003989. In embodiments, the targeted portion of the PAX2 RIC pre-mRNA is in intron 1 or 2. In embodiments, hybridization of an ASO to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of retained intron 1 or 2 and subsequently increases PAX2 protein production. It is understood that the intron numbering may change in reference to a different PAX2 isoform sequence. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_001304569, NM_000278, NM_003990, NM_003988, NM_003987 or NM_003989. One of skill in the art also can determine the sequences of flanking exons in any PAX2 isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_001304569, NM_000278, NM_003990, NM_003988, NM_003987 or NM_003989.

[0016] In embodiments, the CYP24A1 intron numbering corresponds to the mRNA sequence at NM_000782 or NM_001128915. In embodiments, the targeted portion of the CYP24A1 RIC pre-mRNA is in intron 10 and/or 9 or 11. In embodiments, the targeted portion of the CYP24A1 RIC pre-mRNA is in intron 9. In embodiments, the targeted portion of the CYP24A1 RIC pre-mRNA is in intron 11. In embodiments, the percent retained intron can be 23%. In embodiments, the targeted portion of the CYP24A1 RIC pre-mRNA is in intron 10. In embodiments, the percent retained intron can be 50%. In embodiments, hybridization of an ASO to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of retained intron 10 and/or 9 or 11 and subsequently increases CYP24A1 protein production. It is understood that the intron numbering may change in reference to a different CYP24A1 isoform sequence. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the

number provided in reference to the mRNA sequence at NM_000782 or NM_001128915. One of skill in the art also can determine the sequences of flanking exons in any CYP24A1 isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_000782 or NM_001128915.

[0017] In embodiments, the PPARD intron numbering corresponds to the mRNA sequence at NM_006238, NM_177435, NM_001171818, NM_001171819 or NM_001171820. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 2. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 3. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 4. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 5. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 6. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 7. In embodiments, the targeted portion of the PPARD RIC pre-mRNA is in intron 8. In embodiments, hybridization of an ASO to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of a retained intron subsequently increases PPARD protein production. It is understood that the intron numbering may change in reference to a different PPARD isoform sequence. One of skill in the art can determine the corresponding intron number in any isoform based on an intron sequence provided herein or using the number provided in reference to the mRNA sequence at NM_006238, NM_177435, NM_001171818, NM_001171819 or NM_001171820. One of skill in the art also can determine the sequences of flanking exons in any PPARD isoform for targeting using the methods of the invention, based on an intron sequence provided herein or using the intron number provided in reference to the mRNA sequence at NM_006238, NM_177435, NM_001171818, NM_001171819 or NM_001171820.

[0018] In some embodiments, the ASOs disclosed herein target a RIC pre-mRNA transcribed from a *CTNS*, *PAX2*, *CYP24A1* or *PPARD* genomic sequence. In some embodiments, the ASOs disclosed herein target a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA sequence.

[0019] In some embodiments, the ASO targets a sequence of a RIC pre-mRNA transcript encoded by a *CTNS* genomic sequence. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by a *CTNS* genomic sequence comprising retained intron 9 and/or 10. In some embodiments, the ASO targets a RIC pre-mRNA encoded by SEQ ID NO: 3. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 16 or 17. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 16 or 17 comprising a retained intron 9 and/or 10. In some embodiments, the ASOs target SEQ ID NO: 10748 and/or 10734. In some embodiments, the ASO comprises a sequence of any one of SEQ ID NOs: 20-6952.

[0020] In some embodiments, the ASO targets a sequence of a RIC pre-mRNA transcript encoded by a *PAX2* genomic sequence. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by a *PAX2* genomic sequence comprising retained intron 1 or 2. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by SEQ ID NO: 2. In some embodiments, the ASO targets a RIC pre-mRNA transcript of one or more of SEQ ID NO: 10-15. In some embodiments, the ASO targets a RIC pre-mRNA transcript of one or more of SEQ ID NO: 10-15 comprising a retained intron 1 or 2. In some embodiments, the ASOs target SEQ ID NO: 10740 and/or 10738. In some embodiments, the ASO comprises a sequence of any one of SEQ ID NOs: 6953-8623.

[0021] In some embodiments, the ASO targets a sequence of a RIC pre-mRNA transcript encoded by a *CYP24A1* genomic sequence. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by a *CYP24A1* genomic sequence comprising retained intron 10 and/or 9 or 11. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by SEQ ID NO: 4. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 18 or 19. In some embodiments, the ASO targets a RIC pre-mRNA transcript of SEQ ID NO: 18 or 19 comprising a retained intron 10 and/or 9 or 11. In some embodiments, the ASOs target SEQ ID NOs: 10748, 10734, 10746, 10745 and/or 10741. In some embodiments, the ASO comprises a sequence of any one of SEQ ID NOs: 9520-10733.

[0022] In some embodiments, the ASO targets a sequence of a RIC pre-mRNA transcript encoded by a *PPARD* genomic sequence. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by a *PPARD* genomic sequence comprising retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8. In some embodiments, the ASO targets a RIC pre-mRNA transcript encoded by SEQ ID NO: 1. In some embodiments, the ASO targets a RIC pre-mRNA transcript of one or more of SEQ ID NO: 5-9. In some embodiments, the ASO targets a RIC pre-mRNA transcript of one or more of SEQ ID NO: 5-9 comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8. In some embodiments, the ASOs target SEQ ID NO 10736, 10747, 10739, 10743, 10742, 10737, 10735, and/or 10744. In some embodiments, the ASO comprises a sequence of any one of SEQ ID NOs: 20-6952.

[0023] In some embodiments, the ASO targets exon 9 and/or 10 of a *CTNS* RIC pre-mRNA comprising a retained intron 9 and/or 10. In some embodiments, the ASO targets an exon 9 and/or 10 sequence upstream (or 5') from the 5' splice site of a *CTNS* RIC pre-mRNA comprising a retained intron 9 and/or 10.

[0024] In some embodiments, the ASO targets intron 9 and/or 10 in a *CTNS* RIC pre-mRNA comprising a retained intron 9 and/or 10. In some embodiments, the ASO targets an intron 9 and/or 10 sequence downstream (or 3') from the 5' splice site of a *CTNS* RIC pre-mRNA comprising a retained intron 9 and/or 10.

[0025] In some embodiments, the ASO targets an intron 9 and/or 10 sequence upstream (or 5') from the 3' splice site of a *CTNS* RIC pre-mRNA comprising a retained intron 9 and/or 10.

[0026] In some embodiments, the ASO targets exon 10 and/or 11 in a *CTNS* RIC pre-mRNA comprising a

retained intron 9 and/or 10. In some embodiments, the ASO targets an exon 10 and/or 11 sequence downstream (or 3') from the 3' splice site of a *CTNS* RIC pre-mRNA comprising a retained intron 9 and/or 10.

[0027] In some embodiments, the ASO targets exon 1 or 2 of a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2. In some embodiments, the ASO targets an exon 1 or 2 sequence upstream (or 5') from the 5' splice site of a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2.

[0028] In some embodiments, the ASO targets intron 1 or 2 in a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2. In some embodiments, the ASO targets an intron 1 or 2 sequence downstream (or 3') from the 5' splice site of a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2.

[0029] In some embodiments, the ASO targets an intron 1 or 2 sequence upstream (or 5') from the 3' splice site of a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2.

[0030] In some embodiments, the ASO targets exon 2 or 3 in a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2. In some embodiments, the ASO targets an exon 2 or 3 sequence downstream (or 3') from the 3' splice site of a *PAX2* RIC pre-mRNA comprising a retained intron 1 or 2.

[0031] In some embodiments, the ASO targets exon 10 and/or 9 or 11 of a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11. In some embodiments, the ASO targets an exon 10 and/or 9 or 11 sequence upstream (or 5') from the 5' splice site of a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11.

[0032] In some embodiments, the ASO targets intron 10 and/or 9 or 11 in a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11. In some embodiments, the ASO targets an intron 10 and/or 9 or 11 sequence downstream (or 3') from the 5' splice site of a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11.

[0033] In some embodiments, the ASO targets an intron 10 and/or 9 or 11 sequence upstream (or 5') from the 3' splice site of a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11.

[0034] In some embodiments, the ASO targets exon 11 and/or 10 or 12 in a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11. In some embodiments, the ASO targets an exon 11 and/or 10 or 12 sequence downstream (or 3') from the 3' splice site of a *CYP24A1* RIC pre-mRNA comprising a retained intron 10 and/or 9 or 11.

[0035] In some embodiments, the ASO targets exon 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8 of a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8. In some embodiments, the ASO targets an exon 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8 sequence upstream (or 5') from the 5' splice site of a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8.

[0036] In some embodiments, the ASO targets intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8 in a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8. In some embodiments, the ASO targets an intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8 sequence downstream (or 3') from the 5' splice site of a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8.

[0037] In some embodiments, the ASO targets an intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8 sequence upstream (or 5') from the 3' splice site of a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8.

[0038] In some embodiments, the ASO targets exon 4 or 5 or 6 or 7 or 8 and/or 3 or 4 or 5 or 6 or 7 or 8 or 9 in a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8. In some embodiments, the ASO targets an exon 4 or 5 or 6 or 7 or 8 and/or 3 or 4 or 5 or 6 or 7 or 8 or 9 sequence downstream (or 3') from the 3' splice site of a *PPARD* RIC pre-mRNA comprising a retained intron 3 or 4 or 5 or 6 or 7 and/or 2 or 3 or 4 or 5 or 6 or 7 or 8.

Protein Expression

[0039] In embodiments, the methods described herein are used to increase the production of a functional protein. As used herein, the term "functional" refers to the amount of activity or function of a protein that is necessary to eliminate any one or more symptoms of a treated condition. In embodiments, the methods are used to increase the production of a partially functional protein. As used herein, the term "partially functional" refers to any amount of activity or function of the protein that is less than the amount of activity or function that is necessary to eliminate or prevent any one or more symptoms of a disease or condition. In some embodiments, a partially functional protein or RNA will have at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, 85%, at least 90%, or at least 95% less activity relative to the fully functional protein or RNA.

[0040] In embodiments, the method is a method of increasing the expression of the protein by cells of a subject having a RIC pre-mRNA encoding the protein, wherein the subject has a condition described herein caused by a deficient amount of activity of a protein described herein. In some embodiments, the deficient amount of the protein is caused by haploinsufficiency of the protein. In such an embodiment, the subject has a first allele encoding a functional protein, and a second allele from which the protein is not produced. In another such embodiment, the subject has a first allele encoding a functional protein, and a second allele encoding a nonfunctional protein. In another such embodiment, the subject has a first allele encoding a functional protein, and a second allele encoding a partially functional protein. In any of these embodiments, the antisense oligomer binds to a targeted portion of the RIC pre-mRNA transcribed from the first allele (encoding functional protein), thereby inducing constitutive splicing of the retained

intron from the RIC pre-mRNA, and causing an increase in the level of mature mRNA encoding functional protein, and an increase in the expression of the protein in the cells of the subject.

[0041] In embodiments, the subject has a first allele encoding a functional protein, and a second allele encoding a partially functional protein, and the antisense oligomer binds to a targeted portion of the RIC pre-mRNA transcribed from the first allele or a targeted portion of the RIC pre-mRNA transcribed from the second allele (encoding partially functional protein), thereby inducing constitutive splicing of the retained intron from the RIC pre-mRNA, and causing an increase in the level of mature mRNA encoding the protein, and an increase in the expression of functional or partially functional protein in the cells of the subject.

[0042] In related embodiments, the method is a method of using an ASO to increase the expression of a protein or functional RNA. In embodiments, an ASO is used to increase the expression of a protein described herein in cells of a subject having a RIC pre-mRNA encoding the protein, wherein the subject has a deficiency in the amount or function of the protein.

[0043] In embodiments, the RIC pre-mRNA transcript that encodes the protein that is causative of the disease or condition is targeted by the ASOs described herein. In some embodiments, a RIC pre-mRNA transcript that encodes a protein that is not causative of the disease is targeted by the ASOs. For example, a disease that is the result of a mutation or deficiency of a first protein in a particular pathway may be ameliorated by targeting a RIC pre-mRNA that encodes a second protein, thereby increasing production of the second protein. In some embodiments, the function of the second protein is able to compensate for the mutation or deficiency of the first protein (which is causative of the disease or condition).

[0044] In embodiments, the subject has:

a. a first mutant allele from which

i) the protein is produced at a reduced level compared to production from a wild-type allele,

ii) the protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

iii) the protein or functional RNA is not produced; and

b.a second mutant allele from which

i) the protein is produced at a reduced level compared to production from a wild-type allele,

ii) the protein is produced in a form having reduced function compared to an equivalent wild-type protein, or

iii) the protein is not produced, and

wherein the RIC pre-mRNA is transcribed from the first allele and/or the second allele. In these embodiments, the ASO binds to a targeted portion of the RIC pre-mRNA transcribed from the first allele or the second allele, thereby inducing constitutive splicing of the retained intron from the RIC pre-mRNA, and causing an increase in the level of mRNA encoding a protein and an increase in the expression of the target protein or functional RNA in the cells of the subject. In these embodiments, the target protein or functional RNA having an increase in expression level resulting from the constitutive splicing of the retained intron from the RIC pre-mRNA is either in a form having reduced function compared to the equivalent wild-type protein (partially-functional), or having full function compared to the equivalent wild-type protein (fully-functional).

[0045] In embodiments, the level of mRNA encoding a protein described herein is increased 1.1 to 10-fold, when compared to the amount of mRNA encoding the protein that is produced in a control cell, *e.g.*, one that is not treated with the antisense oligomer or one that is treated with an antisense oligomer that does not bind to the targeted portion of the RIC pre-mRNA.

[0046] In embodiments, the condition caused by a deficient amount or activity of a protein is not a condition caused by alternative or aberrant splicing of the retained intron to which the ASO is targeted. In embodiments, the condition caused by a deficient amount or activity of the protein is not a condition caused by alternative or aberrant splicing of any retained intron in a RIC pre-mRNA encoding the protein. In embodiments, alternative or aberrant splicing may occur in a pre-mRNA transcribed from the gene, however the compositions and methods of the invention do not prevent or correct this alternative or aberrant splicing.

[0047] In embodiments, a subject treated using the methods of the invention expresses a partially functional protein from one allele, wherein the partially functional protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, or a partial gene deletion. In embodiments, a subject treated using the methods of the invention expresses a nonfunctional protein from one allele, wherein the nonfunctional protein is caused by a frameshift mutation, a nonsense mutation, a missense mutation, a partial gene deletion, in one allele. In embodiments, a subject treated using the methods of the invention has a whole gene deletion, in one allele.

Use of TANGO for Increasing Protein Expression

[0048] As described above, in embodiments, Targeted Augmentation of Nuclear Gene Output (TANGO) is used in the methods of the invention to increase expression of a protein. In these embodiments, a retained-intron-containing pre-mRNA (RIC pre-mRNA) encoding a protein is present in the nucleus of a cell. Cells having a RIC pre-mRNA that comprises a retained intron, an exon flanking the 5' splice site, and an exon flanking the 3' splice site, encoding the protein, are contacted with antisense oligomers (ASOs) that are complementary to a targeted portion of the RIC pre-mRNA. Hybridization of the ASOs

to the targeted portion of the RIC pre-mRNA results in enhanced splicing at the splice site (5' splice site or 3' splice site) of the retained intron and subsequently increases target protein production.

[0049] The terms "pre-mRNA," and "pre-mRNA transcript" may be used interchangeably and refer to any pre-mRNA species that contains at least one intron. In embodiments, pre-mRNA or pre-mRNA transcripts comprise a 5'-7-methylguanosine cap and/or a poly-A tail. In embodiments, pre-mRNA or pre-mRNA transcripts comprise both a 5'-7-methylguanosine cap and a poly-A tail. In some embodiments, the pre-mRNA transcript does not comprise a 5'-7-methylguanosine cap and/or a poly-A tail. A pre-mRNA transcript is a non-productive messenger RNA (mRNA) molecule if it is not translated into a protein (or transported into the cytoplasm from the nucleus).

[0050] As used herein, a "retained-intron-containing pre-mRNA" ("RIC pre-mRNA") is a pre-mRNA transcript that contains at least one retained intron. The RIC pre-mRNA contains a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and encodes the target protein. An "RIC pre-mRNA encoding a target protein" is understood to encode the target protein when fully spliced. A "retained intron" is any intron that is present in a pre-mRNA transcript when one or more other introns, such as an adjacent intron, encoded by the same gene have been spliced out of the same pre-mRNA transcript. In some embodiments, the retained intron is the most abundant intron in RIC pre-mRNA encoding the target protein. In embodiments, the retained intron is the most abundant intron in a population of RIC pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of RIC pre-mRNAs comprises two or more retained introns. In embodiments, an antisense oligomer targeted to the most abundant intron in the population of RIC pre-mRNAs encoding the target protein induces splicing out of two or more retained introns in the population, including the retained intron to which the antisense oligomer is targeted or binds. In embodiments, a mature mRNA encoding the target protein is thereby produced. The terms "mature mRNA," and "fully-spliced mRNA," are used interchangeably herein to describe a fully processed mRNA encoding a target protein (*e.g.*, mRNA that is exported from the nucleus into the cytoplasm and translated into target protein) or a fully processed functional RNA. The term "productive mRNA," also can be used to describe a fully processed mRNA encoding a target protein. In embodiments, the targeted region is in a retained intron that is the most abundant intron in a RIC pre-mRNA encoding the protein.

[0051] As used herein, the term "comprise" or variations thereof such as "comprises" or "comprising" are to be read to indicate the inclusion of any recited feature (*e.g.* in the case of an antisense oligomer, a defined nucleobase sequence) but not the exclusion of any other features. Thus, as used herein, the term "comprising" is inclusive and does not exclude additional, unrecited features (*e.g.* in the case of an antisense oligomer, the presence of additional, unrecited nucleobases).

[0052] In embodiments of any of the compositions and methods provided herein, "comprising" may be replaced with "consisting essentially of " or "consisting of." The phrase "consisting essentially of" is

used herein to require the specified feature(s) (*e.g.* nucleobase sequence) as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term "consisting" is used to indicate the presence of the recited feature (*e.g.* nucleobase sequence) alone (so that in the case of an antisense oligomer consisting of a specified nucleobase sequence, the presence of additional, unrecited nucleobases is excluded).

[0053] In embodiments, the targeted region is in a retained intron that is the second most abundant intron in a RIC pre-mRNA encoding a protein described herein. For example, the second most abundant retained intron may be targeted rather than the most abundant retained intron due to the uniqueness of the nucleotide sequence of the second most abundant retained intron, ease of ASO design to target a particular nucleotide sequence, and/or amount of increase in protein production resulting from targeting the intron with an ASO. In embodiments, the retained intron is the second most abundant intron in a population of RIC pre-mRNAs transcribed from the gene encoding the target protein in a cell, wherein the population of RIC pre-mRNAs comprises two or more retained introns. In embodiments, an antisense oligomer targeted to the second most abundant intron in the population of RIC pre-mRNAs encoding the target protein induces splicing out of two or more retained introns in the population, including the retained intron to which the antisense oligomer is targeted or binds. In embodiments, fully-spliced (mature) RNA encoding the target protein is thereby produced.

[0054] In embodiments, an ASO is complementary to a targeted region that is within a non-retained intron in a RIC pre-mRNA. In embodiments, the targeted portion of the RIC pre-mRNA is within: the region +6 to +100 relative to the 5' splice site of the non-retained intron; or the region -16 to -100 relative to the 3' splice site of the non-retained intron. In embodiments, the targeted portion of the RIC pre-mRNA is within the region +100 relative to the 5' splice site of the non-retained intron to -100 relative to the 3' splice site of the non-retained intron. As used to identify the location of a region or sequence, "within" is understood to include the residues at the positions recited. For example, a region +6 to +100 includes the residues at positions +6 and +100. In embodiments, fully-spliced (mature) RNA encoding the target protein is thereby produced.

[0055] In embodiments, the retained intron of the RIC pre-mRNA is an inefficiently spliced intron. As used herein, "inefficiently spliced" may refer to a relatively low frequency of splicing at a splice site adjacent to the retained intron (5' splice site or 3' splice site) as compared to the frequency of splicing at another splice site in the RIC pre-mRNA. The term "inefficiently spliced" may also refer to the relative rate or kinetics of splicing at a splice site, in which an "inefficiently spliced" intron may be spliced or removed at a slower rate as compared to another intron in a RIC pre-mRNA.

[0056] In embodiments, the 9-nucleotide sequence at -3e to -1e of the exon flanking the 5' splice site and +1 to +6 of the retained intron is identical to the corresponding wild-type sequence. In embodiments, the 16 nucleotide sequence at -15 to -1 of the retained intron and +1e of the exon flanking the 3' splice site is

identical to the corresponding wild-type sequence. As used herein, the "wild-type sequence" refers to the nucleotide sequence for a gene in the published reference genome deposited in the NCBI repository of biological and scientific information (operated by National Center for Biotechnology Information, National Library of Medicine, 8600 Rockville Pike, Bethesda, MD USA 20894). Also used herein, a nucleotide position denoted with an "e" indicates the nucleotide is present in the sequence of an exon (*e.g.*, the exon flanking the 5' splice site or the exon flanking the 3' splice site).

[0057] The methods involve contacting cells with an ASO that is complementary to a portion of a pre-mRNA encoding a protein described herein, resulting in increased expression of the protein. As used herein, "contacting" or administering to cells refers to any method of providing an ASO in immediate proximity with the cells such that the ASO and the cells interact. A cell that is contacted with an ASO will take up or transport the ASO into the cell. The method involves contacting a condition or disease-associated or condition or disease-relevant cell with an ASO. In some embodiments, the ASO may be further modified or attached (*e.g.*, covalently attached) to another molecule to target the ASO to a cell type, enhance contact between the ASO and the condition or disease-associated or condition or disease-relevant cell, or enhance uptake of the ASO.

[0058] As used herein, the term "increasing protein production" or "increasing expression of a target protein" means enhancing the amount of protein that is translated from an mRNA in a cell. A "target protein" may be any protein for which increased expression/production is desired.

[0059] In embodiments, contacting a cell that expresses a RIC pre-mRNA with an ASO that is complementary to a targeted portion of the RIC pre-mRNA transcript results in a measurable increase in the amount of a protein (*e.g.*, a target protein) encoded by the pre-mRNA. Methods of measuring or detecting production of a protein will be evident to one of skill in the art and include any known method, for example, Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA.

[0060] In embodiments, contacting cells with an ASO that is complementary to a targeted portion of an RIC pre-mRNA transcript results in an increase in the amount of a protein produced by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In embodiments, the total amount of a protein produced by the cell to which the antisense oligomer was contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or

at least about 10-fold, compared to the amount of target protein produced by an control compound. A control compound can be, for example, an oligonucleotide that is not complementary to the targeted portion of the RIC pre-mRNA.

[0061] In some embodiments, contacting cells with an ASO that is complementary to a targeted portion of an RIC pre-mRNA transcript results in an increase in the amount of mRNA encoding a protein, including the mature mRNA encoding the target protein. In some embodiments, the amount of mRNA encoding a protein, or the mature mRNA encoding the protein, is increased by at least 10, 20, 30, 40, 50, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, or 1000%, compared to the amount of the protein produced by a cell in the absence of the ASO/absence of treatment. In embodiments, the total amount of the mRNA encoding a protein, or the mature mRNA encoding a protein produced in the cell to which the antisense oligomer was contacted is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold compared to the amount of mature RNA produced in an untreated cell, *e.g.*, an untreated cell or a cell treated with a control compound. A control compound can be, for example, an oligonucleotide that is not complementary to the targeted portion of the RIC pre-mRNA.

Constitutive Splicing of a Retained Intron from a RIC pre-mRNA

[0062] The methods and antisense oligonucleotide compositions provided herein are useful for increasing the expression of a protein in cells, for example, in a subject having a condition described herein caused by a deficiency in the amount or activity of a protein described herein, by increasing the level of mRNA encoding the protein, or the mature mRNA encoding the protein. In particular, the methods and compositions as described herein induce the constitutive splicing of a retained intron from an RIC pre-mRNA transcript encoding the protein, thereby increasing the level of mRNA encoding the protein, or the mature mRNA encoding the protein and increasing the expression of the protein.

[0063] Constitutive splicing of a retained intron from a RIC pre-mRNA correctly removes the retained intron from the RIC pre-mRNA, wherein the retained intron has wild-type splice sequences. Constitutive splicing, as used herein, does not encompass splicing of a retained intron from a RIC pre-mRNA transcribed from a gene or allele having a mutation that causes alternative splicing or aberrant splicing of a pre-mRNA transcribed from the gene or allele. For example, constitutive splicing of a retained intron, as induced using the methods and antisense oligonucleotides provided herein, does not correct aberrant

splicing in or influence alternative splicing of a pre-mRNA to result in an increased expression of a target protein or functional RNA.

[0001] In embodiments, constitutive splicing correctly removes a retained intron from an RIC pre-mRNA, wherein the RIC pre-mRNA is transcribed from a wild-type gene or allele, or a polymorphic gene or allele, that encodes a fully-functional target protein or functional RNA, and wherein the gene or allele does not have a mutation that causes alternative splicing or aberrant splicing of the retained intron.

[0064] In some embodiments, constitutive splicing of a retained intron from an RIC pre-mRNA encoding a protein correctly removes a retained intron from an RIC pre-mRNA encoding the protein, wherein the RIC pre-mRNA is transcribed from a gene or allele from which the target gene or functional RNA is produced at a reduced level compared to production from a wild-type allele, and wherein the gene or allele does not have a mutation that causes alternative splicing or aberrant splicing of the retained intron. In these embodiments, the correct removal of the constitutively spliced retained intron results in production of target protein or functional RNA that is functional when compared to an equivalent wild-type protein or functional RNA.

[0065] In other embodiments, constitutive splicing correctly removes a retained intron from an RIC pre-mRNA, wherein the RIC pre-mRNA is transcribed from a gene or allele that encodes a target protein or functional RNA produced in a form having reduced function compared to an equivalent wild-type protein or functional RNA, and wherein the gene or allele does not have a mutation that causes alternative splicing or aberrant splicing of the retained intron. In these embodiments, the correct removal of the constitutively spliced retained intron results in production of partially functional target protein, or functional RNA that is partially functional when compared to an equivalent wild-type protein or functional RNA.

[0066] "Correct removal" of the retained intron by constitutive splicing refers to removal of the entire intron, without removal of any part of an exon.

[0067] In embodiments, an antisense oligomer as described herein or used in any method described herein does not increase the amount of mRNA encoding a protein or the amount of a protein by modulating alternative splicing or aberrant splicing of a pre-mRNA transcribed from the gene. Modulation of alternative splicing or aberrant splicing can be measured using any known method for analyzing the sequence and length of RNA species, *e.g.*, by RT-PCR and using methods described elsewhere herein and in the literature. In embodiments, modulation of alternative or aberrant splicing is determined based on an increase or decrease in the amount of the spliced species of interest of at least 10% or 1.1-fold. In embodiments, modulation is determined based on an increase or decrease at a level that is at least 10% to 100% or 1.1 to 10-fold, as described herein regarding determining an increase in mRNA encoding the protein in the methods of the invention.

[0068] In embodiments, the method is a method wherein the RIC pre-mRNA was produced by partial splicing of a wild-type pre-mRNA. In embodiments, the method is a method wherein the RIC pre-mRNA was produced by partial splicing of a full-length wild-type pre-mRNA. In embodiments, the RIC pre-mRNA was produced by partial splicing of a full-length pre-mRNA. In these embodiments, a full-length pre-mRNA may have a polymorphism in a splice site of the retained intron that does not impair correct splicing of the retained intron as compared to splicing of the retained intron having the wild-type splice site sequence.

[0069] In embodiments, the mRNA encoding a protein is a full-length mature mRNA, or a wild-type mature mRNA. In these embodiments, a full-length mature mRNA may have a polymorphism that does not affect the activity of the target protein or the functional RNA encoded by the mature mRNA, as compared to the activity of the protein encoded by the wild-type mature mRNA.

Antisense Oligomers

[0070] One aspect of the present disclosure is a composition comprising antisense oligomers that enhances splicing by binding to a targeted portion of an RIC pre-mRNA. As used herein, the terms "ASO" and "antisense oligomer" are used interchangeably and refer to an oligomer such as a polynucleotide, comprising nucleobases, that hybridizes to a target nucleic acid (*e.g.*, an RIC pre-mRNA) sequence by Watson-Crick base pairing or wobble base pairing (G-U). The ASO may have exact sequence complementary to the target sequence or near complementarity (*e.g.*, sufficient complementarity to bind the target sequence and enhancing splicing at a splice site). ASOs are designed so that they bind (hybridize) to a target nucleic acid (*e.g.*, a targeted portion of a pre-mRNA transcript) and remain hybridized under physiological conditions. Typically, if they hybridize to a site other than the intended (targeted) nucleic acid sequence, they hybridize to a limited number of sequences that are not a target nucleic acid (to a few sites other than a target nucleic acid). Design of an ASO can take into consideration the occurrence of the nucleic acid sequence of the targeted portion of the pre-mRNA transcript or a sufficiently similar nucleic acid sequence in other locations in the genome or cellular pre-mRNA or transcriptome, such that the likelihood the ASO will bind other sites and cause "off-target" effects is limited. Any antisense oligomers known in the art, for example in PCT Application No. PCT/US2014/054151, published as WO 2015/035091, titled "Reducing Nonsense-Mediated mRNA Decay," can be used to practice the methods described herein.

[0071] In some embodiments, ASOs "specifically hybridize" to or are "specific" to a target nucleic acid or a targeted portion of a RIC pre-mRNA. Typically such hybridization occurs with a Tm substantially greater than 37°C, preferably at least 50°C, and typically between 60°C to approximately 90°C. Such hybridization preferably corresponds to stringent hybridization conditions. At a given ionic strength and pH, the Tm is the temperature at which 50% of a target sequence hybridizes to a complementary oligonucleotide.

[0072] Oligomers, such as oligonucleotides, are "complementary" to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides. A double-stranded polynucleotide can be "complementary" to another polynucleotide, if hybridization can occur between one of the strands of the first polynucleotide and the second. Complementarity (the degree to which one polynucleotide is complementary with another) is quantifiable in terms of the proportion (*e.g.*, the percentage) of bases in opposing strands that are expected to form hydrogen bonds with each other, according to generally accepted base-pairing rules. The sequence of an antisense oligomer (ASO) need not be 100% complementary to that of its target nucleic acid to hybridize. In certain embodiments, ASOs can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an ASO in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered together or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. Percent complementarity of an ASO with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656).

[0073] An ASO need not hybridize to all nucleobases in a target sequence and the nucleobases to which it does hybridize may be contiguous or noncontiguous. ASOs may hybridize over one or more segments of a pre-mRNA transcript, such that intervening or adjacent segments are not involved in the hybridization event (*e.g.*, a loop structure or hairpin structure may be formed). In certain embodiments, an ASO hybridizes to noncontiguous nucleobases in a target pre-mRNA transcript. For example, an ASO can hybridize to nucleobases in a pre-mRNA transcript that are separated by one or more nucleobase(s) to which the ASO does not hybridize.

[0074] The ASOs described herein comprise nucleobases that are complementary to nucleobases present in a target portion of a RIC pre-mRNA. The term ASO embodies oligonucleotides and any other oligomeric molecule that comprises nucleobases capable of hybridizing to a complementary nucleobase on a target mRNA but does not comprise a sugar moiety, such as a peptide nucleic acid (PNA). The ASOs may comprise naturally-occurring nucleotides, nucleotide analogs, modified nucleotides, or any combination of two or three of the preceding. The term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" includes nucleotides with modified or substituted sugar groups and/or having a modified backbone. In some embodiments, all of the nucleotides of the ASO are modified nucleotides. Chemical modifications of ASOs or components of ASOs that are compatible with the methods and compositions described herein will be evident to one of

skill in the art and can be found, for example, in U.S. Patent No. 8,258,109 B2, U.S. Patent No. 5,656,612, U.S. Patent Publication No. 2012/0190728, and Dias and Stein, Mol. Cancer Ther. 2002, 1, 347-355, herein incorporated by reference in their entirety.

[0075] The nucleobase of an ASO may be any naturally occurring, unmodified nucleobase such as adenine, guanine, cytosine, thymine and uracil, or any synthetic or modified nucleobase that is sufficiently similar to an unmodified nucleobase such that it is capable of hydrogen bonding with a nucleobase present on a target pre-mRNA. Examples of modified nucleobases include, without limitation, hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethoylcytosine.

[0076] The ASOs described herein also comprise a backbone structure that connects the components of an oligomer. The term "backbone structure" and "oligomer linkages" may be used interchangeably and refer to the connection between monomers of the ASO. In naturally occurring oligonucleotides, the backbone comprises a 3'-5' phosphodiester linkage connecting sugar moieties of the oligomer. The backbone structure or oligomer linkages of the ASOs described herein may include (but are not limited to) phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoramidate, and the like. See *e.g.*, LaPlanche et al., Nucleic Acids Res. 14:9081 (1986); Stec et al., J. Am. Chem. Soc. 106:6077 (1984), Stein et al., Nucleic Acids Res. 16:3209 (1988), Zon et al., Anti Cancer Drug Design 6:539 (1991); Zon et al., Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al., U.S. Pat. No. 5,151,510; Uhlmann and Peyman, Chemical Reviews 90:543 (1990). In some embodiments, the backbone structure of the ASO does not contain phosphorous but rather contains peptide bonds, for example in a peptide nucleic acid (PNA), or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. In some embodiments, the backbone modification is a phosphorothioate linkage. In some embodiments, the backbone modification is a phosphoramidate linkage.

[0077] In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is random. In embodiments, the stereochemistry at each of the phosphorus internucleotide linkages of the ASO backbone is controlled and is not random. For example, U.S. Pat. App. Pub. No. 2014/0194610, "Methods for the Synthesis of Functionalized Nucleic Acids," incorporated herein by reference, describes methods for independently selecting the handedness of chirality at each phosphorous atom in a nucleic acid oligomer. In embodiments, an ASO used in the methods of the invention comprises an ASO having phosphorus internucleotide linkages that are not random. In embodiments, a composition used in the methods of the invention comprises a pure diastereomeric ASO. In embodiments, a composition used in the methods of the invention comprises an ASO that has diastereomeric purity of at least about 90%, at least about 91%, at least about 92%, at least about 93%, at

least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, about 100%, about 90% to about 100%, about 91% to about 100%, about 92% to about 100%, about 93% to about 100%, about 94% to about 100%, about 95% to about 100%, about 96% to about 100%, about 97% to about 100%, about 98% to about 100%, or about 99% to about 100%.

[0078] In embodiments, the ASO has a nonrandom mixture of Rp and Sp configurations at its phosphorus internucleotide linkages. For example, it has been suggested that a mix of Rp and Sp is required in antisense oligonucleotides to achieve a balance between good activity and nuclease stability (Wan, et al., 2014, "Synthesis, biophysical properties and biological activity of second generation antisense oligonucleotides containing chiral phosphorothioate linkages," Nucleic Acids Research 42(22): 13456-13468, incorporated herein by reference). In embodiments, an ASO used in the methods of the invention comprises about 5-100% Rp, at least about 5% Rp, at least about 10% Rp, at least about 15% Rp, at least about 20% Rp, at least about 25% Rp, at least about 30% Rp, at least about 35% Rp, at least about 40% Rp, at least about 45% Rp, at least about 50% Rp, at least about 55% Rp, at least about 60% Rp, at least about 65% Rp, at least about 70% Rp, at least about 75% Rp, at least about 80% Rp, at least about 85% Rp, at least about 90% Rp, or at least about 95% Rp, with the remainder Sp, or about 100% Rp. In embodiments, an ASO used in the methods of the invention comprises about 10% to about 100% Rp, about 15% to about 100% Rp, about 20% to about 100% Rp, about 25% to about 100% Rp, about 30% to about 100% Rp, about 35% to about 100% Rp, about 40% to about 100% Rp, about 45% to about 100% Rp, about 50% to about 100% Rp, about 55% to about 100% Rp, about 60% to about 100% Rp, about 65% to about 100% Rp, about 70% to about 100% Rp, about 75% to about 100% Rp, about 80% to about 100% Rp, about 85% to about 100% Rp, about 90% to about 100% Rp, or about 95% to about 100% Rp, about 20% to about 80% Rp, about 25% to about 75% Rp, about 30% to about 70% Rp, about 40% to about 60% Rp, or about 45% to about 55% Rp, with the remainder Sp.

[0079] In embodiments, an ASO used in the methods of the invention comprises about 5-100% Sp, at least about 5% Sp, at least about 10% Sp, at least about 15% Sp, at least about 20% Sp, at least about 25% Sp, at least about 30% Sp, at least about 35% Sp, at least about 40% Sp, at least about 45% Sp, at least about 50% Sp, at least about 55% Sp, at least about 60% Sp, at least about 65% Sp, at least about 70% Sp, at least about 75% Sp, at least about 80% Sp, at least about 85% Sp, at least about 90% Sp, or at least about 95% Sp, with the remainder Rp, or about 100% Sp. In embodiments, an ASO used in the methods of the invention comprises about 10% to about 100% Sp, about 15% to about 100% Sp, about 20% to about 100% Sp, about 25% to about 100% Sp, about 30% to about 100% Sp, about 35% to about 100% Sp, about 40% to about 100% Sp, about 45% to about 100% Sp, about 50% to about 100% Sp, about 55% to about 100% Sp, about 60% to about 100% Sp, about 65% to about 100% Sp, about 70% to about 100% Sp, about 75% to about 100% Sp, about 80% to about 100% Sp, about 85% to about 100% Sp, about 90% to about 100% Sp, or about 95% to about 100% Sp, about 20% to about 80% Sp, about

25% to about 75% Sp, about 30% to about 70% Sp, about 40% to about 60% Sp, or about 45% to about 55% Sp, with the remainder Rp.

[0080] Any of the ASOs described herein may contain a sugar moiety that comprises ribose or deoxyribose, as present in naturally occurring nucleotides, or a modified sugar moiety or sugar analog, including a morpholine ring. Non-limiting examples of modified sugar moieties include 2' substitutions such as 2'-O-methyl (2′-O-Me), 2'-O-methoxyethyl (2'MOE), 2'-O-aminoethyl, 2'F; N3'->P5' phosphoramidate, 2'dimethylaminooxyethoxy, 2'dimethylaminoethoxyethoxy, 2'-guanidinidium, 2'-O-guanidinium ethyl, carbamate modified sugars, and bicyclic modified sugars. In some embodiments, the sugar moiety modification is selected from 2′-O-Me, 2'F, and 2'MOE. In some embodiments, the sugar moiety modification is an extra bridge bond, such as in a locked nucleic acid (LNA). In some embodiments the sugar analog contains a morpholine ring, such as phosphorodiamidate morpholino (PMO). In some embodiments, the sugar moiety comprises a ribofuransyl or 2'deoxyribofuransyl modification. In some embodiments, the sugar moiety comprises 2'4'-constrained 2'O-methyloxyethyl (cMOE) modifications. In some embodiments, the sugar moiety comprises cEt 2', 4' constrained 2'-O ethyl BNA modifications. In some embodiments, the sugar moiety comprises tricycloDNA (tcDNA) modifications. In some embodiments, the sugar moiety comprises ethylene nucleic acid (ENA) modifications. In some embodiments, the sugar moiety comprises MCE modifications. Modifications are known in the art and described in the literature, *e.g.*, by Jarver, et al., 2014, "A Chemical View of Oligonucleotides for Exon Skipping and Related Drug Applications," Nucleic Acid Therapeutics 24(1): 37-47, incorporated by reference for this purpose herein.

[0081] In some examples, each monomer of the ASO is modified in the same way, for example each linkage of the backbone of the ASO comprises a phosphorothioate linkage or each ribose sugar moiety comprises a 2'O-methyl modification. Such modifications that are present on each of the monomer components of an ASO are referred to as "uniform modifications." In some examples, a combination of different modifications may be desired, for example, an ASO may comprise a combination of phosphorodiamidate linkages and sugar moieties comprising morpholine rings (morpholinos). Combinations of different modifications to an ASO are referred to as "mixed modifications" or "mixed chemistries."

[0082] In some embodiments, the ASO comprises one or more backbone modification. In some embodiments, the ASO comprises one or more sugar moiety modification. In some embodiments, the ASO comprises one or more backbone modification and one or more sugar moiety modification. In some embodiments, the ASO comprises 2'MOE modifications and a phosphorothioate backbone. In some embodiments, the ASO comprises a phosphorodiamidate morpholino (PMO). In some embodiments, the ASO comprises a peptide nucleic acid (PNA). Any of the ASOs or any component of an ASO (*e.g.*, a nucleobase, sugar moiety, backbone) described herein may be modified in order to achieve desired

properties or activities of the ASO or reduce undesired properties or activities of the ASO. For example, an ASO or one or more component of any ASO may be modified to enhance binding affinity to a target sequence on a pre-mRNA transcript; reduce binding to any non-target sequence; reduce degradation by cellular nucleases (i.e., RNase H); improve uptake of the ASO into a cell and/or into the nucleus of a cell; alter the pharmacokinetics or pharmacodynamics of the ASO; and modulate the half-life of the ASO.

[0083] In some embodiments, the ASOs are comprised of 2'-O-(2-methoxyethyl) (MOE) phosphorothioate-modified nucleotides. ASOs comprised of such nucleotides are especially well-suited to the methods disclosed herein; oligomers having such modifications have been shown to have significantly enhanced resistance to nuclease degradation and increased bioavailability, making them suitable, for example, for oral delivery in some embodiments described herein. See *e.g.*, Geary et al., J Pharmacol Exp Ther. 2001; 296(3):890-7; Geary et al., J Pharmacol Exp Ther. 2001; 296(3):898-904.

[0084] Methods of synthesizing ASOs will be known to one of skill in the art. Alternatively or in addition, ASOs may be obtained from a commercial source.

[0085] Unless specified otherwise, the left-hand end of single-stranded nucleic acid (*e.g.*, pre-mRNA transcript, oligonucleotide, ASO, etc.) sequences is the 5′ end and the left-hand direction of single or double-stranded nucleic acid sequences is referred to as the 5′ direction. Similarly, the right-hand end or direction of a nucleic acid sequence (single or double stranded) is the 3′ end or direction. Generally, a region or sequence that is 5′ to a reference point in a nucleic acid is referred to as "upstream," and a region or sequence that is 3′ to a reference point in a nucleic acid is referred to as "downstream." Generally, the 5′ direction or end of an mRNA is where the initiation or start codon is located, while the 3′ end or direction is where the termination codon is located. In some aspects, nucleotides that are upstream of a reference point in a nucleic acid may be designated by a negative number, while nucleotides that are downstream of a reference point may be designated by a positive number. For example, a reference point (*e.g.*, an exon-exon junction in mRNA) may be designated as the "zero" site, and a nucleotide that is directly adjacent and upstream of the reference point is designated "minus one," *e.g.*, "-1," while a nucleotide that is directly adjacent and downstream of the reference point is designated "plus one," *e.g.*, "+1."

[0086] In other embodiments, the ASOs are complementary to (and bind to) a targeted portion of a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is downstream (in the 3' direction) of the 5' splice site of the retained intron in a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA (*e.g.*, the direction designated by positive numbers relative to the 5' splice site) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region 1 to +4000, 1 to +3500, 1 to +3000, 1 to +2500, 1 to +2000, 1 to +1500, 1 to +1000, 1 to +500, +2 to +4000, +2 to +3500, +2 to +3000, +2 to +2500, +2 to +2000, +2 to +1500, +2 to +1000, +2 to +500, +2 to +400, +2 to +300, +2 to +200, +6 to +4000, +6 to +3500, +6 to +3000, +6 to +2500,

+6 to +2000, +6 to +1500, +6 to +1000, +6 to +500, +6 to +400, +6 to +300, +6 to +200, or +6 to +100 relative to the 5' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides +1 to +5 relative to the 5' splice site (the first five nucleotides located downstream of the 5' splice site). In some embodiments, the ASOs may be complementary to a targeted portion of a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region between nucleotides +6 and +50 relative to the 5' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region +6 to +90, +6 to +80, +6 to +70, +6 to +60, +6 to +50, +6 to +40, +6 to +30, or +6 to +20 relative to 5' splice site of the retained intron.

[0087] In some embodiments, the ASOs are complementary to a targeted region of a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is upstream (5' relative) of the 3' splice site of the retained intron in an RIC pre-mRNA (*e.g.*, in the direction designated by negative numbers) (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region -16 to -4000, -16 to -3000, -16 to -2000, -16 to -1000, -16 to -500, -16 to -400, -16 to -300, -6 to -200, or -16 to -100 relative to the 3' splice site of the retained intron. In some embodiments, the ASO is not complementary to nucleotides -1 to -15 relative to the 3' splice site (the first 15 nucleotides located upstream of the 3' splice site). In some embodiments, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region -16 to -50 relative to the 3' splice site of the retained intron. In some aspects, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region -16 to -90, -16 to -80, -16 to -70, -16 to -60, -16 to -50, -16 to -40, or -16 to -30 relative to 3' splice site of the retained intron.

[0088] In embodiments, the targeted portion of the RIC pre-mRNA is within the region +100 relative to the 5' splice site of the retained intron to -100 relative to the 3' splice site of the retained intron.

[0089] In some embodiments, the ASOs are complementary to a targeted portion of a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the exon flanking the 5' splice site (upstream) of the retained intron (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion of the RIC pre-mRNA that is within the region +2e to -4e in the exon flanking the 5' splice site of the retained intron. In some embodiments, the ASOs are not complementary to nucleotides -1e to -3e relative to the 5' splice site of the retained intron. In some embodiments, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region -4e to-100e, -4e to -90e, -4e to -80e, -4e to -70e, -4e to -60e, -4e to -50e, -4 to -40e, -4e to -30e, or -4e to -20e relative to the 5' splice site of the retained intron.

[0090] In some embodiments, the ASOs are complementary to a targeted portion of a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the exon flanking the 3' splice site (downstream) of

the retained intron (FIG. 1). In some embodiments, the ASOs are complementary to a targeted portion to the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region +2e to -4e in the exon flanking the 3' splice site of the retained intron. In some embodiments, the ASOs are not complementary to nucleotide +1e relative to the 3' splice site of the retained intron. In some embodiments, the ASOs are complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA that is within the region+2e to +100e, +2e to +90e, +2e to +80e, +2e to +70e, +2e to +60e, +2e to +50e, +2e to +40e, +2e to +30e, or +2 to +20e relative to the 3' splice site of the retained intron. The ASOs may be of any length suitable for specific binding and effective enhancement of splicing. In some embodiments, the ASOs consist of 8 to 50 nucleobases. For example, the ASO may be 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, or 50 nucleobases in length. In some embodiments, the ASOs consist of more than 50 nucleobases. In some embodiments, the ASO is from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, 12 to 15 nucleobases, 13 to 50 nucleobases, 13 to 40 nucleobases, 13 to 35 nucleobases, 13 to 30 nucleobases, 13 to 25 nucleobases, 13 to 20 nucleobases, 14 to 50 nucleobases, 14 to 40 nucleobases, 14 to 35 nucleobases, 14 to 30 nucleobases, 14 to 25 nucleobases, 14 to 20 nucleobases, 15 to 50 nucleobases, 15 to 40 nucleobases, 15 to 35 nucleobases, 15 to 30 nucleobases, 15 to 25 nucleobases, 15 to 20 nucleobases, 20 to 50 nucleobases, 20 to 40 nucleobases, 20 to 35 nucleobases, 20 to 30 nucleobases, 20 to 25 nucleobases, 25 to 50 nucleobases, 25 to 40 nucleobases, 25 to 35 nucleobases, or 25 to 30 nucleobases in length. In some embodiments, the ASOs are 30 nucleotides in length. In some embodiments, the ASOs are 29 nucleotides in length. In some embodiments, the ASOs are 28 nucleotides in length. In some embodiments, the ASOs are 27 nucleotides in length. In some embodiments, the ASOs are 26 nucleotides in length. In some embodiments, the ASOs are 25 nucleotides in length. In some embodiments, the ASOs are 24 nucleotides in length. In some embodiments, the ASOs are 23 nucleotides in length. In some embodiments, the ASOs are 22 nucleotides in length. In some embodiments, the ASOs are 21 nucleotides in length. In some embodiments, the ASOs are 20 nucleotides in length. In some embodiments, the ASOs are 19 nucleotides in length. In some embodiments, the ASOs are 18 nucleotides in length. In some embodiments, the ASOs are 17 nucleotides in length. In some embodiments, the ASOs are 16 nucleotides in length. In some embodiments, the ASOs are 15 nucleotides in length. In some embodiments, the ASOs are 14 nucleotides in length. In some embodiments, the ASOs are 13 nucleotides in

length. In some embodiments, the ASOs are 12 nucleotides in length. In some embodiments, the ASOs are 11 nucleotides in length. In some embodiments, the ASOs are 10 nucleotides in length.

[0091] In some embodiments, two or more ASOs with different chemistries but complementary to the same targeted portion of the RIC pre-mRNA are used. In some embodiments, two or more ASOs that are complementary to different targeted portions of the RIC pre-mRNA are used.

[0092] In embodiments, the antisense oligonucleotides of the invention are chemically linked to one or more moieties or conjugates, *e.g.*, a targeting moiety or other conjugate that enhances the activity or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, a lipid moiety, *e.g.*, as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, *e.g.*, dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Oligonucleotides comprising lipophilic moieties, and preparation methods have been described in the published literature. In embodiments, the antisense oligonucleotide is conjugated with a moiety including, but not limited to, an abasic nucleotide, a polyether, a polyamine, a polyamide, a peptides, a carbohydrate, *e.g.*, N-acetylgalactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (*e.g.*, mannose-6-phosphate), a lipid, or a polyhydrocarbon compound. Conjugates can be linked to one or more of any nucleotides comprising the antisense oligonucleotide at any of several positions on the sugar, base or phosphate group, as understood in the art and described in the literature, *e.g.*, using a linker. Linkers can include a bivalent or trivalent branched linker. In embodiments, the conjugate is attached to the 3' end of the antisense oligonucleotide. Methods of preparing oligonucleotide conjugates are described, *e.g.*, in U.S. Pat. No. 8,450,467, "Carbohydrate conjugates as delivery agents for oligonucleotides," incorporated by reference herein.

[0093] In some embodiments, the nucleic acid to be targeted by an ASO is an RIC pre-mRNA expressed in a cell, such as a eukaryotic cell. In some embodiments, the term "cell" may refer to a population of cells. In some embodiments, the cell is in a subject. In some embodiments, the cell is isolated from a subject. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is a condition or disease-relevant cell or a cell line. In some embodiments, the cell is *in vitro* (*e.g.*, in cell culture).

Pharmaceutical Compositions

[0094] Pharmaceutical compositions or formulations comprising the antisense oligonucleotide of the described compositions and for use in any of the described methods can be prepared according to conventional techniques well known in the pharmaceutical industry and described in the published literature. In embodiments, a pharmaceutical composition or formulation for treating a subject comprises an effective amount of any antisense oligomer as described above, or a pharmaceutically acceptable salt, solvate, hydrate or ester thereof, and a pharmaceutically acceptable diluent. The antisense oligomer of a pharmaceutical formulation may further comprise a pharmaceutically acceptable excipient, diluent or carrier.

[0095] Pharmaceutically acceptable salts are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, etc., and are commensurate with a reasonable benefit/risk ratio. (See, e.g., S. M. Berge, et al., J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference for this purpose. The salts can be prepared in situ during the final isolation and purification of the compounds, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other documented methodologies such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[0096] In embodiments, the compositions are formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. In embodiments, the compositions are formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers. In embodiments, a pharmaceutical formulation or composition of the present invention includes, but is not limited to, a solution, emulsion, microemulsion, foam or liposome-containing formulation (e.g., cationic or noncationic liposomes).

[0097] The pharmaceutical composition or formulation of the present invention may comprise one or more penetration enhancer, carrier, excipients or other active or inactive ingredients as appropriate and well known to those of skill in the art or described in the published literature. In embodiments, liposomes also include sterically stabilized liposomes, e.g., liposomes comprising one or more specialized lipids. These specialized lipids result in liposomes with enhanced circulation lifetimes. In embodiments, a sterically stabilized liposome comprises one or more glycolipids or is derivatized with one or more

hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. In embodiments, a surfactant is included in the pharmaceutical formulation or compositions. The use of surfactants in drug products, formulations and emulsions is well known in the art. In embodiments, the present invention employs a penetration enhancer to effect the efficient delivery of the antisense oligonucleotide, e.g., to aid diffusion across cell membranes and /or enhance the permeability of a lipophilic drug. In embodiments, the penetration enhancers is a surfactant, fatty acid, bile salt, chelating agent, or non-chelating nonsurfactant.

[0098] In embodiments, the pharmaceutical formulation comprises multiple antisense oligonucleotides. In embodiments, the antisense oligonucleotide is administered in combination with another drug or therapeutic agent. In embodiments, the antisense oligonucleotide is administered with one or more agents capable of promoting penetration of the subject antisense oligonucleotide across the blood-brain barrier by any method known in the art. For example, delivery of agents by administration of an adenovirus vector to motor neurons in muscle tissue is described in U.S. Pat. No. 6,632,427, "Adenoviral-vector-mediated gene transfer into medullary motor neurons," incorporated herein by reference. Delivery of vectors directly to the brain, e.g., the striatum, the thalamus, the hippocampus, or the substantia nigra, is described, e.g., in U.S. Pat. No. 6,756,523, "Adenovirus vectors for the transfer of foreign genes into cells of the central nervous system particularly in brain," incorporated herein by reference.

[0099] In embodiments, the antisense oligonucleotides are linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. In embodiments, the antisense oligonucleotide is coupled to a substance, known in the art to promote penetration or transport across the blood-brain barrier, e.g., an antibody to the transferrin receptor. In embodiments, the antisense oligonucleotide is linked with a viral vector, e.g., to render the antisense compound more effective or increase transport across the blood-brain barrier. In embodiments, osmotic blood brain barrier disruption is assisted by infusion of sugars, e.g., meso erythritol, xylitol, D(+) galactose, D(+) lactose, D(+) xylose, dulcitol, myo-inositol, L(-) fructose, D(-) mannitol, D(+) glucose, D(+) arabinose, D(-) arabinose, cellobiose, D(+) maltose, D(+) raffinose, L(+) rhamnose, D(+) melibiose, D(-) ribose, adonitol, D(+) arabitol, L(-) arabitol, D(+) fucose, L(-) fucose, D(-) lyxose, L(+) lyxose, and L(-) lyxose, or amino acids, e.g., glutamine, lysine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, leucine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and taurine. Methods and materials for enhancing blood brain barrier penetration are described, e.g., in U.S. Pat. No. 4,866,042, "Method for the delivery of genetic material across the blood brain barrier," U.S. Pat. No. 6,294,520, "Material for passage through the blood-brain barrier," and U.S. Pat. No. 6,936,589, "Parenteral delivery systems," each incorporated herein by reference.

[00100] In embodiments, the antisense oligonucleotides of the invention are chemically linked to one or more moieties or conjugates, e.g., a targeting moiety or other conjugate that enhances the activity or cellular uptake of the oligonucleotide. Such moieties include, but are not limited to, a lipid moiety, e.g.,

as a cholesterol moiety, a cholesteryl moiety, an aliphatic chain, e.g., dodecandiol or undecyl residues, a polyamine or a polyethylene glycol chain, or adamantane acetic acid. Oligonucleotides comprising lipophilic moieties, and preparation methods have been described in the published literature. In embodiments, the antisense oligonucleotide is conjugated with a moiety including, but not limited to, an abasic nucleotide, a polyether, a polyamine, a polyamide, a peptides, a carbohydrate, e.g., N-acetylgalactosamine (GalNAc), N-Ac-Glucosamine (GluNAc), or mannose (e.g., mannose-6-phosphate), a lipid, or a polyhydrocarbon compound. Conjugates can be linked to one or more of any nucleotides comprising the antisense oligonucleotide at any of several positions on the sugar, base or phosphate group, as understood in the art and described in the literature, e.g., using a linker. Linkers can include a bivalent or trivalent branched linker. In embodiments, the conjugate is attached to the 3' end of the antisense oligonucleotide. Methods of preparing oligonucleotide conjugates are described, *e.g.*, in U.S. Pat. No. 8,450,467, "Carbohydrate conjugates as delivery agents for oligonucleotides," incorporated by reference herein.

Treatment of Subjects

[00101] Any of the compositions provided herein may be administered to an individual. "Individual" may be used interchangeably with "subject" or "patient." An individual may be a mammal, for example a human or animal such as a non-human primate, a rodent, a rabbit, a rat, a mouse, a horse, a donkey, a goat, a cat, a dog, a cow, a pig, or a sheep. In embodiments, the individual is a human. In embodiments, the individual is a fetus, an embryo, or a child. In other embodiments, the individual may be another eukaryotic organism, such as a plant. In some embodiments, the compositions provided herein are administered to a cell *ex vivo*.

[00102] In some embodiments, the compositions provided herein are administered to an individual as a method of treating a disease or disorder. In some embodiments, the individual has a genetic disease, such as any of the diseases described herein. In some embodiments, the individual is at risk of having the disease, such as any of the diseases described herein. In some embodiments, the individual is at increased risk of having a disease or disorder caused by insufficient amount of a protein or insufficient activity of a protein. If an individual is "at an increased risk" of having a disease or disorder caused insufficient amount of a protein or insufficient activity of a protein, the method involves preventative or prophylactic treatment. For example, an individual may be at an increased risk of having such a disease or disorder because of family history of the disease. Typically, individuals at an increased risk of having such a disease or disorder benefit from prophylactic treatment (*e.g.*, by preventing or delaying the onset or progression of the disease or disorder).

[00103] Suitable routes for administration of ASOs of the present invention may vary depending on cell type to which delivery of the ASOs is desired. Multiple tissues and organs can be affected in a condition described herein, with the kidney being the most significantly affected tissue. The ASOs of the present

invention may be administered to patients parenterally, for example, by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection. In embodiments, delivery is to the kidney. In embodiments, a fetus is treated in utero, e.g., by administering the ASO composition to the fetus directly or indirectly (e.g., via the mother).

Methods of identifying additional ASOs that enhance splicing

[00104] Also within the scope of the present invention are methods for identifying (determining) additional ASOs that enhance splicing of an RIC pre-mRNA, specifically at the target intron. ASOs that specifically hybridize to different nucleotides within the target region of the pre-mRNA may be screened to identify (determine) ASOs that improve the rate and/or extent of splicing of the target intron. In some embodiments, the ASO may block or interfere with the binding site(s) of a splicing repressor(s)/silencer. Any method known in the art may be used to identify (determine) an ASO that when hybridized to the target region of the intron results in the desired effect (*e.g.*, enhanced splicing, protein or functional RNA production). These methods also can be used for identifying ASOs that enhance splicing of the retained intron by binding to a targeted region in an exon flanking the retained intron, or in a non-retained intron. An example of a method that may be used is provided below.

[00105] A round of screening, referred to as an ASO "walk" may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. For example, the ASOs used in the ASO walk can be tiled every 5 nucleotides from approximately 100 nucleotides upstream of the 5' splice site of the retained intron (*e.g.*, a portion of sequence of the exon located upstream of the target/retained intron) to approximately 100 nucleotides downstream of the 5' splice site of the target/retained intron and/or from approximately 100 nucleotides upstream of the 3' splice site of the retained intron to approximately 100 nucleotides downstream of the 3' splice site of the target/retained intron (*e.g.*, a portion of sequence of the exon located downstream of the target/retained intron). For example, a first ASO of 15 nucleotides in length may be designed to specifically hybridize to nucleotides +6 to +20 relative to the 5' splice site of the target/retained intron. A second ASO is designed to specifically hybridize to nucleotides +11 to +25 relative to the 5' splice site of the target/retained intron. ASOs are designed as such spanning the target region of the pre-mRNA. In embodiments, the ASOs can be tiled more closely, *e.g.*, every 1, 2, 3, or 4 nucleotides. Further, the ASOs can be tiled from 100 nucleotides downstream of the 5' splice site, to 100 nucleotides upstream of the 3' splice site.

[00106] One or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region) are delivered, for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA (e.g., the RIC pre-mRNA described elsewhere herein). The splicing-inducing effects of each of the ASOs may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described herein (see "Identification of intron-retention events"). A reduction or absence of the RT-PCR

product produced using the primers spanning the splice junction in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target intron has been enhanced. In some embodiments, the splicing efficiency, the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00107] A second round of screening, referred to as an ASO "micro-walk" may be performed using ASOs that have been designed to hybridize to a target region of a pre-mRNA. The ASOs used in the ASO micro-walk are tiled every 1 nucleotide to further refine the nucleotide acid sequence of the pre-mRNA that when hybridized with an ASO results in enhanced splicing.

[00108] Regions defined by ASOs that promote splicing of the target intron are explored in greater detail by means of an ASO "micro-walk", involving ASOs spaced in 1-nt steps, as well as longer ASOs, typically 18-25 nt.

[00109] As described for the ASO walk above, the ASO micro-walk is performed by delivering one or more ASOs, or a control ASO (an ASO with a scrambled sequence, sequence that is not expected to hybridize to the target region), for example by transfection, into a disease-relevant cell line that expresses the target pre-mRNA. The splicing-inducing effects of each of the ASOs may be assessed by any method known in the art, for example by reverse transcriptase (RT)-PCR using primers that span the splice junction, as described herein (see "Identification of intron-retention events"). A reduction or absence of the RT-PCR product produced using the primers spanning the splice junction in ASO-treated cells as compared to in control ASO-treated cells indicates that splicing of the target intron has been enhanced. In some embodiments, the splicing efficiency, the ratio of spliced to unspliced pre-mRNA, the rate of splicing, or the extent of splicing may be improved using the ASOs described herein. The amount of protein or functional RNA that is encoded by the target pre-mRNA can also be assessed to determine whether each ASO achieved the desired effect (*e.g.*, enhanced protein production). Any method known in the art for assessing and/or quantifying protein production, such as Western blotting, flow cytometry, immunofluorescence microscopy, and ELISA, can be used.

[00110] ASOs that when hybridized to a region of a pre-mRNA result in enhanced splicing and increased protein production may be tested *in vivo* using animal models, for example transgenic mouse models in which the full-length human gene has been knocked-in or in humanized mouse models of disease. Suitable routes for administration of ASOs may vary depending on the disease and/or the cell types to which delivery of the ASOs is desired. ASOs may be administered, for example, by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection. Following

administration, the cells, tissues, and/or organs of the model animals may be assessed to determine the effect of the ASO treatment by for example evaluating splicing (efficiency, rate, extent) and protein production by methods known in the art and described herein. The animal models may also be any phenotypic or behavioral indication of the disease or disease severity.

[00111] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[00112] The present invention will be more specifically illustrated by the following Examples. However, it should be understood that the present invention is not limited by these examples in any manner.

Example 1: Identification of intron retention events in transcripts by RNAseq using next generation sequencing

[00113] Whole transcriptome shotgun sequencing was carried out using next generation sequencing to reveal a snapshot of transcripts produced by a gene described herein to identify intron-retention events. For this purpose, polyA+ RNA from nuclear and cytoplasmic fractions of renal epithelial cells was isolated and cDNA libraries constructed using Illumina's TruSeq Stranded mRNA library Prep Kit. The libraries were pair-end sequenced resulting in 100-nucleotide reads that were mapped to the human genome (Feb. 2009, GRCh37/hg19 assembly) The mapped reads were visualized using the UCSC genome browser (operated by the UCSC Genome Informatics Group (Center for Biomolecular Science & Engineering, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064) and described by, e.g., Rosenbloom, et al., 2015, "The UCSC Genome Browser database: 2015 update," Nucleic Acids Research 43, Database Issue, doi: 10.1093/nar/gku1177) and the coverage and number of reads were inferred by the peak signals. The height of the peaks indicates the level of expression given by the density of the reads in a particular region. A schematic representation of the gene (drawn to scale) was provided by the UCSC genome browser (below the read signals) so that peaks can be matched to the exonic and intronic regions. Based on this display, we identified introns that have high read density in the nuclear fraction of renal epithelial cells, but have very low to no reads in the cytoplasmic fraction of these cells (see Table 1 for percent intron retention (PIR) data for introns identified in genes described herein). This indicated that these introns were retained and that the intron-containing transcripts remain in the nucleus, and suggested that these retained RIC pre-mRNAs are non-productive, as they were not exported out to the cytoplasm.

Example 2: Identification of intron retention events in gene transcripts by RNAseq using next generation sequencing for retained introns not yet identified

[00114] Whole transcriptome shotgun sequencing was carried out using next generation sequencing to reveal a snapshot of transcripts produced by a gene described herein to identify unknown intron-retention events. For this purpose, polyA+ RNA from nuclear and cytoplasmic fractions of renal epithelial cells were isolated and cDNA libraries were constructed using Illumina's TruSeq Stranded mRNA library Prep Kit. The libraries were pair-end sequenced resulting in 100-nucleotide reads that were mapped to the human genome (Feb. 2009, GRCh37/hg19 assembly) The mapped reads were visualized using the UCSC genome browser (operated by the UCSC Genome Informatics Group (Center for Biomolecular Science & Engineering, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064) and described by, e.g., Rosenbloom, et al., 2015, "The UCSC Genome Browser database: 2015 update,"

Nucleic Acids Research 43, Database Issue, doi: 10.1093/nar/gku1177) and the coverage and number of reads can be inferred by the peak signals. The height of the peaks indicated the level of expression given by the density of the reads in a particular region. A schematic representation of the gene (drawn to scale) was provided by the UCSC genome browser (below the read signals) so that peaks could be matched to the gene exonic and intronic regions. Based on this display, retained introns were inferred as those that have high read density in the nuclear fraction of renal epithelial cells, but have very low to no reads in the cytoplasmic fraction of these cells. This indicated that the introns are retained and that the retained intron-containing transcripts remained in the nucleus, and suggests that these retained RIC pre-mRNAs are non-productive, as they are not exported out to the cytoplasm.

Example 3: Design of ASO-walk targeting a retained intron

[00115] An ASO walk was designed to target a retained intron using the method described herein. A region immediately downstream of the intron 5' splice site, *e.g.*, spanning nucleotides +6 to +69 and a region immediately upstream of intron 3' splice site, *e.g.*, spanning nucleotides -16 to -79 of the intron was targeted with 2'-O-Me RNA, PS backbone, 18-mer ASOs shifted by 5-nucleotide intervals. Table 1 lists exemplary ASOs that were designed and their target sequences.

Table 1

Gene SEQ ID NO.	Gene Symbol	Pre-mRNA (RNA Accession Number) SEQ ID NO.	ASOs SEQ ID NOs.	Retained Intron (% Retention)	Target Sequence SEQ ID NO.
PPARD SEQ ID NO. 1	5467	PPARD:NM_006238 SEQ ID NO. 5	20-276	Intron 3	10736
			277-519	Intron 4	10747
			520-772	Intron 5	10739
			773-931	Intron 6	10743
			932-1622	Intron 7	10742
		PPARD:NM_177435 SEQ ID NO. 6	1623-1879	Intron 3	10736
			1880-2122	Intron 4	10747
			2123-2374	Intron 5	10739
			2375-2655	Intron 6	10737
		PPARD:NM_001171818 SEQ ID NO. 7	2656-3346	Intron 8	10742
			3347-3603	Intron 4	10736
			3604-3846	Intron 5	10747
			3847-4098	Intron 6	10739
			4099-4258	Intron 7	10743
		PPARD:NM_001171819 SEQ ID NO. 8	5259-4488	Intron 2	10735
			4489-4731	Intron 3	10747

			4732-4983	Intron 4	10739
			4984-5143	Intron 5	10743
			5144-5834	Intron 6	10742
		PPARD:NM_001171820 SEQ ID NO. 9	5835-6101	Intron 3	10744
			6102-6261	Intron 4	10743
			6262-6952	Intron 5	10742
PAX2 SEQ ID NO. 2	5076	PAX2:NM_001304569 SEQ ID NO. 10	6953-7173	Intron 2	10738
		PAX2:NM_000278 SEQ ID NO. 11	7174-7463	Intron 1	10740
		PAX2:NM_003990 SEQ ID NO. 12	7464-7753	Intron 1 (N/A)	10740
		PAX2:NM_003988 SEQ ID NO. 13	7754-8043	Intron 1	10740
		PAX2:NM_003987 SEQ ID NO. 14	8044-8333	Intron 1	10740
		PAX2:NM_003989 SEQ ID NO. 15	8334-8623	Intron 1	10740
CTNS SEQ ID NO. 3	1497	CTNS:NM_004937 SEQ ID NO. 16	8624-8846	Intron 9 (10%)	10748
			8847-9071	Intron 10	10734
		CTNS:NM_001031681 SEQ ID NO. 17	9072-9294	Intron 9 (10%)	10748
			9295-9519	Intron 10	10734
CYP24A1 SEQ ID NO. 4	1591	CYP24A1:NM_000782 SEQ ID NO. 18	9520-9595	Intron 10 (23%)	10746
			9596-10068	Intron 11 (50%)	10745
		CYP24A1:NM_001128915 SEQ ID NO. 19	10069-10260	Intron 9	10741
			10261-10733	Intron 10	10745

Example 4: Improved splicing efficiency via ASO-targeting of a retained intron increases *CTNS* transcript levels

[00116] To determine whether an increase in expression of CTNS could be achieved by improving splicing efficiency of a retained intron using ASOs, the methods described herein were used. ARPE-19 cells were mock-transfected, or transfected with CTNS targeting ASOs, or a non-targeting ASO control, independently, using RNAiMAX (Invitrogen) delivery reagents. Experiments were performed using 80 nM ASOs for 24 hrs (FIG. 3B and C). Taqman qPCR results showed that several

targeting ASOs increase CTNS gene transcript level compared to the mock-transfected. Ct values from CTNS targeting-ASO-transfected cells are normalized to RPL32 and plotted relative to the normalized qPCR product from mock-treated cells. Results of this analysis indicated that several CTNS targeting ASOs increase gene transcript levels. These results show that inducing splicing of a retained intron in the gene using ASOs leads to an increase in gene expression. Altogether, these results show that improving the splicing efficiency of a rate limiting intron in the CTNS gene using ASOs led to an increase in CTNS gene expression.

Example 5: Improved splicing efficiency via ASO-targeting of a retained intron increases *CYP24A1* transcript levels

[00117] To determine whether an increase in expression of CYP24A1 could be achieved by improving splicing efficiency of a retained intron using ASOs, the methods described herein were used. ARPE-19 cells were mock-transfected, or transfected with CYP24A1 targeting ASOs, or a non-targeting ASO control, independently, using RNAiMAX (Invitrogen) delivery reagents. Experiments were performed using 80 nM ASOs for 24 hrs (FIG. 4B and C). Taqman qPCR results showed that several targeting ASOs increase CYP24A1 gene transcript level compared to the mock-transfected. Ct values from CYP24A1 targeting-ASO-transfected cells are normalized to RPL32 and plotted relative to the normalized qPCR product from mock-treated cells. Results of this analysis indicated that several CYP24A1 targeting ASOs increase gene transcript levels. These results show that inducing splicing of a retained intron in the gene using ASOs leads to an increase in gene expression. Altogether, these results show that improving the splicing efficiency of a rate limiting intron in the CYP24A1 gene using ASOs led to an increase in CYP24A1 gene expression.

Example 6: Improved splicing efficiency via ASO-targeting of a retained intron increases *PPARD* transcript levels

[00118] To determine whether an increase in expression of PPARD could be achieved by improving splicing efficiency of a retained intron using ASOs, the methods described herein were used. ARPE-19 cells were mock-transfected, or transfected with PPARD targeting ASOs, or a non-targeting ASO control, independently, using RNAiMAX (Invitrogen) delivery reagents. Experiments were performed using 80 nM ASOs for 24 hrs (FIG. 6A and B, FIG. 6E and F). Taqman qPCR results showed that several targeting ASOs increase PPARD gene transcript level compared to the mock-transfected. Ct values from PPARD targeting-ASO-transfected cells are normalized to RPL32 and plotted relative to the normalized qPCR product from mock-treated cells. Results of this analysis indicated that several PPARD targeting ASOs increase gene transcript levels. These results show that inducing splicing of a retained intron in the gene using ASOs leads to an increase in gene expression.

Altogether, these results show that improving the splicing efficiency of a rate limiting intron in the PPARD gene using ASOs led to an increase in PPARD gene expression.

Example 7: Improved splicing efficiency via ASO-targeting of a retained intron increases transcript levels

[00119] To determine whether an increase in expression of a target gene could be acheived by improving intron splicing efficiency with ASOs we used the method described herein. ARPE-19 cells, a human retinal epithelium cell line (American Type Culture Collection (ATCC), USA), or Huh-7, a human hepatoma cell line (NIBIOHN, Japan), or SK-N-AS, a human neuroblastoma cell line (ATCC) were mock-transfected, or transfected with the targeting ASOs described in FIGs. 3-6 and Table 1. Cells were transfected using Lipofectamine RNAiMax transfection reagent (Thermo Fisher) according to vendor's specifications. Briefly, ASOs were plated in 96-well tissue culture plates and combined with RNAiMax diluted in Opti-MEM. Cells were detached using trypsin and resuspended in full medium, and approximately 25,000 cells were added the ASO-transfection mixture. Transfection experiments were carried out in triplicate plate replicates. Final ASO concentration was 80 nM. Media was changed 6h post-transfection, and cells harvested at 24h, using the Cells-to-Ct lysis reagent, supplemented with DNAse (Thermo Fisher), according to vendor's specifications. cDNA was generated with Cells-to-Ct RT reagents (Thermo Fisher) according to vendor's specifications. To quantify the amount of splicing at the intron of interest, quantitative PCR was carried out using Taqman assays with probes spanning the corresponding exon-exon junction (Thermo Fisher) of the reetained intron listed in Table 1. Taqman assays were carried out according to vendor's specifications, on a QuantStudio 7 Flex Real-Time PCR system (Thermo Fisher). Target gene assay values were normalized to RPL32 (deltaCt) and plate-matched mock transfected samples (delta-delta Ct), generating fold-change over mock quantitation (2^-(delta-deltaCt). Average fold-change over mock of the three plate replicates was plotted (FIG. 3C, FIG. 4C, FIG. 6B and FIG. 6F). Several ASOs were identified that increase the target gene expression, implying an increase in splicing at that target intron. Together with whole transcriptome data showing retention of the target intron (FIGs. 3-6), these results confirm that ASOs can improve the splicing efficiency of a rate limiting intron.

CLAIMS

What is claimed is:

1. A method of treating a kidney disease in a subject in need thereof by increasing the expression of a target protein or functional RNA by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site, and wherein the RIC pre-mRNA encodes the target protein or functional RNA, the method comprising contacting the cells of the subject with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein or functional RNA, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject.
2. The method of claim 1, wherein the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.
3. A method of increasing expression of a target protein by cells having a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and wherein the RIC pre-mRNA encodes the target protein, the method comprising contacting the cells with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein, thereby increasing the level of mRNA encoding the target protein, and increasing the expression of the target protein in the cells, wherein the target protein is cystinosin, protein paired box gene 2 protein, protein cytochrome P450 family 24, subfamily A, polypeptide 1, or peroxisome proliferator activated receptor delta.
4. The method of claim 1 or 2, wherein the target protein is cystinosin, protein paired box gene 2 protein, protein cytochrome P450 family 24, subfamily A, polypeptide 1, or peroxisome proliferator activated receptor delta.
5. The method of claim 1 or 2, wherein the target protein or the functional RNA is a compensating protein or a compensating functional RNA that functionally augments or replaces a target protein or functional RNA that is deficient in amount or activity in the subject.
6. The method of claim 3, wherein the cells are in or from a subject having a condition caused by a deficient amount or activity of the target protein.
7. The method of any one of claims 1 to 6, wherein the deficient amount of the target protein is caused by haploinsufficiency of the target protein, wherein the subject has a first allele encoding a functional target protein, and a second allele from which the target protein is not produced, or a second allele

encoding a nonfunctional target protein, and wherein the antisense oligomer binds to a targeted portion of a RIC pre-mRNA transcribed from the first allele.

8. The method of any one of claims 1 to 6, wherein the subject has a condition caused by a disorder resulting from a deficiency in the amount or function of the target protein, wherein the subject has
   (a) a first mutant allele from which
      (i) the target protein is produced at a reduced level compared to production from a wild-type allele,
      (ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
      (iii)the target protein is not produced, and
   (b) a second mutant allele from which
      (i) the target protein is produced at a reduced level compared to production from a wild-type allele,
      (ii) the target protein is produced in a form having reduced function compared to an equivalent wild-type protein, or
      (iii)the target protein is not produced, and
   wherein when the subject has a first mutant allele a(iii), the second mutant allele is b(i) or b(ii), and wherein when the subject has a second mutant allele b(iii), the first mutant allele is a(i) or a(ii), and wherein the RIC pre-mRNA is transcribed from either the first mutant allele that is a(i) or a(ii), and/or the second allele that is b(i)(ii) or b(ii).
9. The method of claim 8, wherein the target protein is produced in a form having reduced function compared to the equivalent wild-type protein.
10. The method of claim 8, wherein the target protein is produced in a form that is fully-functional compared to the equivalent wild-type protein.
11. The method of any one of claims 1 to 10, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron.
12. The method of any one of claims 1 to 10, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +69 relative to the 5' splice site of the retained intron to -79 relative to the 3' splice site of the retained intron.
13. The method of any one of claims 1 to 12, wherein the target protein is cystinosin.
14. The method of claim 13, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.
15. The method of claim 13 or 14, wherein the targeted portion of the RIC pre-mRNA comprises a

sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 8624-10068.

16. The method of any one of claims 13 to 15, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748 or SEQ ID NO 10734.
17. The method of any one of claims 13 to 16, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 8624-10068.
18. The method of any one of claims 13 to 17, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 16 or SEQ ID NO 17.
19. The method of any one of claims 13 to 18, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 3.
20. The method of any one of claims 1 to 12, wherein the target protein is paired box gene 2 protein.
21. The method of claim 20, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.
22. The method of claim 20 or 21, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 6953-8623.
23. The method of any one of claims 20 to 22, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10738 or SEQ ID NO 10740.
24. The method of any one of claims 20 to 23, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 6953-8623.
25. The method of any one of claims 20 to 24, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 10-15.
26. The method of any one of claims 20 to 25, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 2.

27. The method of any one of claims 1 to 12, wherein the target protein is protein cytochrome P450 family 24, subfamily A, polypeptide 1.
28. The method of claim 27, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.
29. The method of claim 27 or 28, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 9520-10733.
30. The method of any one of claims 27 to 29, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748, SEQ ID NO 10734, SEQ ID NO 10746, SEQ ID NO 10745 or SEQ ID NO 10741.
31. The method of any one of claims 27 to 30, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 9520-10733.
32. The method of any one of claims 27 to 31, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 16-19.
33. The method of any one of claims 27 to 32, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 4.
34. The method of any one of claims 1 to 12, wherein the target protein is peroxisome proliferator activated receptor delta.
35. The method of claim 34, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.
36. The method of claim 34 or 35, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 20-6952.
37. The method of any one of claims 34 to 36, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10736, SEQ ID NO 10747, SEQ ID NO 10739, SEQ ID NO 10743, SEQ ID NO 10742, SEQ ID NO 10737, SEQ ID NO 10735, or SEQ ID NO 10744.
38. The method of any one of claims 34 to 37, wherein the ASO comprises a sequence with at least about

80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 20-6952.

39. The method of any one of claims 34 to 38, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 5-9.
40. The method of any one of claims 34 to 39, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 1.
41. The method of any one of claims 1 to 10 and 13-40, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within:
    (a) the region +6 to +100 relative to the 5' splice site of the retained intron; or
    (b) the region -16 to -100 relative to the 3' splice site of the retained intron.
42. The composition of any one of claims 1 to 10 and 13-40, wherein the antisense oligomer targets a portion of the RIC pre-mRNA that is within the region about 100 nucleotides downstream of the 5' splice site of the at least one retained intron, to about 100 nucleotides upstream of the 3' splice site of the at least one retained intron
43. The method of any one of claims 1 to 10 and 13-40, wherein the targeted portion of the RIC pre-mRNA is within:
    (a) the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or
    (b) the region +2e to -4e in the exon flanking the 3' splice site of the retained intron.
44. The method of any one of claims 1 to 43, wherein the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating alternative splicing of pre-mRNA transcribed from a gene encoding the functional RNA or target protein.
45. The method of any one of claims 1 to 44, wherein the antisense oligomer does not increase the amount of the target protein or the functional RNA by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or the functional RNA.
46. The method of any one of claims 1 to 45, wherein the RIC pre-mRNA was produced by partial splicing of a full-length pre-mRNA or partial splicing of a wild-type pre-mRNA.
47. The method of any one of claims 1 to 46, wherein the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.
48. The method of any one of claims 1 to 47, wherein the target protein produced is full-length protein, or wild-type protein.
49. The method of any one of claims 1 to 48, wherein the total amount of the mRNA encoding the target protein or functional RNA produced in the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-

fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of the mRNA encoding the target protein or functional RNA produced in a control cell.

50. The method of any one of claims 1 to 49, wherein the total amount of target protein produced by the cell contacted with the antisense oligomer is increased about 1.1 to about 10-fold, about 1.5 to about 10-fold, about 2 to about 10-fold, about 3 to about 10-fold, about 4 to about 10-fold, about 1.1 to about 5-fold, about 1.1 to about 6-fold, about 1.1 to about 7-fold, about 1.1 to about 8-fold, about 1.1 to about 9-fold, about 2 to about 5-fold, about 2 to about 6-fold, about 2 to about 7-fold, about 2 to about 8-fold, about 2 to about 9-fold, about 3 to about 6-fold, about 3 to about 7-fold, about 3 to about 8-fold, about 3 to about 9-fold, about 4 to about 7-fold, about 4 to about 8-fold, about 4 to about 9-fold, at least about 1.1-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 5-fold, or at least about 10-fold, compared to the total amount of target protein produced by a control cell.
51. The method of any one of claims 1 to 50, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.
52. The method of any one of claims 1 to 51, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.
53. The method of any one of claims 1 to 52, wherein the antisense oligomer comprises at least one modified sugar moiety.
54. The method of claim 53, wherein each sugar moiety is a modified sugar moiety.
55. The method of any one of claims 1 to 54, wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

56. The method of any one of claims 1 to 55, wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, complementary to the targeted portion of the RIC pre-mRNA encoding the protein.
57. The method of any one of claims 1 to 56, wherein the cell comprises a population of RIC pre-mRNAs transcribed from the gene encoding the target protein or functional RNA, wherein the population of RIC pre-mRNAs comprises two or more retained introns, and wherein the antisense oligomer binds to the most abundant retained intron in the population of RIC pre-mRNAs.
58. The method of claim 57, whereby the binding of the antisense oligomer to the most abundant retained intron induces splicing out of the two or more retained introns from the population of RIC pre-mRNAs to produce mRNA encoding the target protein or functional RNA.
59. The method of any one of claims 1 to 56, wherein the cell comprises a population of RIC pre-mRNAs transcribed from the gene encoding the target protein or functional RNA, wherein the population of RIC pre-mRNAs comprises two or more retained introns, and wherein the antisense oligomer binds to the second most abundant retained intron in the population of RIC pre-mRNAs.
60. The method of claim 59, whereby the binding of the antisense oligomer to the second most abundant retained intron induces splicing out of the two or more retained introns from the population of RIC pre-mRNAs to produce mRNA encoding the target protein or functional RNA.
61. The method of any one of claims 6 to 60, wherein the condition is a disease or disorder.
62. The method of claim 61, wherein the disease or disorder is a kidney disease.
63. The method of claim 62, wherein the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.
64. The method of claim 63, wherein the target protein and the RIC pre-mRNA are encoded by a gene, wherein the gene is *CTNS, PAX2, CYP24A1* or *PPARD.*
65. The method of any one of claims 1 to 64, wherein the method further comprises assessing protein expression.
66. The method of any one of claims 1 to 65, wherein the subject is a human.
67. The method of any one of claims 1 to 65, wherein the subject is a non-human animal.
68. The method of any one of claims 1 to 66, wherein the subject is a fetus, an embryo, or a child.
69. The method of any one of claims 1 to 67, wherein the cells are *ex vivo.*
70. The method of any one of claims 1 to 67, wherein the antisense oligomer is administered by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject.
71. The method of any one of claims 1 to 70, wherein the 9 nucleotides at -3e to -1e of the exon flanking the 5' splice site and +1 to +6 of the retained intron, are identical to the corresponding wild-type sequence.

72. The method of any one of claims 1 to 71, wherein the 16 nucleotides at -15 to -1 of the retained intron and +1e of the exon flanking the 3' splice site are identical to the corresponding wild-type sequence.
73. An antisense oligomer as used in a method of any one of claims 1 to 72.
74. An antisense oligomer comprising a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 20-10733.
75. A pharmaceutical composition comprising the antisense oligomer of claim 73 or 74 and an excipient.
76. A method of treating a subject in need thereof by administering the pharmaceutical composition of claim 75 by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.
77. A composition comprising an antisense oligomer for use in a method of increasing expression of a target protein or a functional RNA by cells to treat a kidney disease in a subject in need thereof associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject, wherein the antisense oligomer enhances constitutive splicing of a retained intron-containing pre-mRNA (RIC pre-mRNA) encoding the target protein or the functional RNA, wherein the target protein is:
    (a) the deficient protein; or
    (b) a compensating protein which functionally augments or replaces the deficient protein or in the subject; and wherein the functional RNA is:
    (a) the deficient RNA; or
    (b) a compensating functional RNA which functionally augments or replaces the deficient functional RNA in the subject;
    wherein the RIC pre-mRNA comprises a retained intron, an exon flanking the 5' splice site and an exon flanking the 3' splice site, and wherein the retained intron is spliced from the RIC pre-mRNA encoding the target protein or the functional RNA, thereby increasing production or activity of the target protein or the functional RNA in the subject.
78. The composition of claim 77, wherein the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.
79. A composition comprising an antisense oligomer for use in a method of treating a condition associated with a target protein in a subject in need thereof, the method comprising the step of increasing expression of the target protein by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA) comprising a retained intron, an exon flanking the 5' splice site of the retained intron, an exon flanking the 3' splice site of the retained intron, and wherein the RIC pre-mRNA encodes the target protein, the method comprising contacting the cells with the

antisense oligomer, whereby the retained intron is constitutively spliced from the RIC pre-mRNA transcripts encoding the target protein, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein, in the cells of the subject.

80. The composition of any one of claims 77 to 79, wherein the target protein is cystinosin, protein paired box gene 2 protein, protein cytochrome P450 family 24, subfamily A, polypeptide 1, or peroxisome proliferator activated receptor delta.
81. The composition of claim 79, wherein the condition is a disease or disorder.
82. The composition of claim 80, wherein the disease or disorder is a kidney disease.
83. The composition of claim 82, wherein the kidney disease is infantile nephropathic cystinosis, late-onset cystinosis, focal segmental glomerulosclerosis 7, Papillorenal syndrome, or infantile hypercalcemia.
84. The composition of claim 82 or 83, wherein the target protein and RIC pre-mRNA are encoded by a gene, wherein the gene is *CTNS, PAX2, CYP24A1* or *PPARD*.
85. The composition of any one of claims 77 to 84, wherein the antisense oligomer targets a portion of the RIC pre-mRNA that is in the retained intron within the region +6 relative to the 5' splice site of the retained intron to -16 relative to the 3' splice site of the retained intron.
86. The composition of any one of claims 77 to 84, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +69 relative to the 5' splice site of the retained intron to -79 relative to the 3' splice site of the retained intron.
87. The composition of any one of claims 77 to 86, wherein the target protein is cystinosin.
88. The composition of claim 87, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3'of the retained intron.
89. The composition of claim 87 or 88, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 8624-10068.
90. The composition of any one of claims 87 to 89, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748 or SEQ ID NO 10734.
91. The composition of any one of claims 87 to 90, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 8624-10068.
92. The composition of any one of claims 87 to 91, wherein the RIC pre-mRNA comprises a sequence

with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 16 or SEQ ID NO 17.

93. The composition of any one of claims 87 to 92, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 3.
94. The composition of any one of claims 77 to 86, wherein the target protein is paired box gene 2 protein.
95. The composition of claim 94, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.
96. The composition of claim 94 or 95, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 6953-8623.
97. The composition of any one of claims 94 to 96, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10738 or SEQ ID NO 10740.
98. The composition of any one of claims 94 to 97, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 6953-8623.
99. The composition of any one of claims 94 to 98, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 10-15.
100. The composition of any one of claims 94 to 99, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 2.
101. The composition of any one of claims 77 to 86, wherein the target protein is protein cytochrome P450 family 24, subfamily A, polypeptide 1.
102. The composition of claim 101, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.
103. The composition of claim 101 or 102, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs. 9520-10733.
104. The composition of any one of claims 101 to 103, wherein the targeted portion of the RIC pre-

mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10748, SEQ ID NO 10734, SEQ ID NO 10746, SEQ ID NO 10745 or SEQ ID NO 10741.

105. The composition of any one of claims 101 to 104, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 9520-10733.

106. The composition of any one of claims 101 to 105, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 16-19.

107. The composition of any one of claims 101 to 106, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 4.

108. The composition of any one of claims 77 to 86, wherein the target protein is peroxisome proliferator activated receptor delta.

109. The composition of claim 108, wherein the targeted portion of the RIC pre-mRNA is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' splice site of the retained intron.

110. The composition of claim 108 or 109, wherein the targeted portion of the RIC pre-mRNA comprises a sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complimentary to any one of SEQ ID NOs 20-6952.

111. The composition of any one of claims 108 to 110, wherein the targeted portion of the RIC pre-mRNA comprises a sequence with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a region comprising at least 8 contiguous nucleic acids of SEQ ID NO 10736, SEQ ID NO 10747, SEQ ID NO 10739, SEQ ID NO 10743, SEQ ID NO 10742, SEQ ID NO 10737, SEQ ID NO 10735, or SEQ ID NO 10744.

112. The composition of any one of claims 108 to 111, wherein the ASO comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 20-6952.

113. The composition of any one of claims 108 to 112, wherein the RIC pre-mRNA comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs 5-9.

114. The composition of any one of claims 108 to 113, wherein the RIC pre-mRNA is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO 1.

115. The composition of any one of claims 77 to 84 and 87 to 114, wherein the antisense oligomer targets

a portion of the RIC pre-mRNA that is in the retained intron within:

(a) the region +6 to +100 relative to the 5' splice site of the retained intron; or

(b) the region -16 to -100 relative to the 3' splice site of the retained intron.

116. The composition of any one of claims 77 to 84 and 87 to 114, wherein the antisense oligomer targets a portion of the RIC pre-mRNA that is within the region about 100 nucleotides downstream of the 5' splice site of the at least one retained intron, to about 100 nucleotides upstream of the 3' splice site of the at least one retained intron.

117. The composition of any one of claims 77 to 84 and 87 to 114, wherein the targeted portion of the RIC pre-mRNA is within:

(a) the region +2e to -4e in the exon flanking the 5' splice site of the retained intron; or

(b) the region +2e to -4e in the exon flanking the 3' splice site of the retained intron.

118. The composition of any one of claims 77 to 117, wherein the antisense oligomer does not increase the amount of target protein or functional RNA by modulating alternative splicing of the pre-mRNA transcribed from a gene encoding the target protein or functional RNA.

119. The composition of any one of claims 77 to 118, wherein the antisense oligomer does not increase the amount of the functional RNA or functional protein by modulating aberrant splicing resulting from mutation of the gene encoding the target protein or functional RNA.

120. The composition of any one of claims 77 to 119, wherein the RIC pre-mRNA was produced by partial splicing from a full-length pre-mRNA or a wild-type pre-mRNA.

121. The composition of any one of claims 77 to 120, wherein the mRNA encoding the target protein or functional RNA is a full-length mature mRNA, or a wild-type mature mRNA.

122. The composition of any one of claims 77 to 121, wherein the target protein produced is full-length protein, or wild-type protein.

123. The composition of any one of claims 77 to 122, wherein the retained intron is a rate-limiting intron.

124. The composition of any one of claims 77 to 123, wherein said retained intron is the most abundant retained intron in said RIC pre-mRNA.

125. The composition of any one of claims 77 to 123, wherein the retained intron is the second most abundant retained intron in said RIC pre-mRNA.

126. The composition of any one of claims 77 to 125, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

127. The composition of any one of claims 77 to 126, wherein said antisense oligomer is an antisense oligonucleotide.

128. The composition of any one of claims 77 to 127, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

129. The composition of any one of claims 77 to 128, wherein the antisense oligomer comprises at least one modified sugar moiety.
130. The composition of claim 129, wherein each sugar moiety is a modified sugar moiety.
131. The composition of any one of claims 77 to 130, wherein the antisense oligomer consists of from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.
132. The composition of any one of claims 77 to 131, wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or is 100% complementary to the targeted portion of the RIC pre-mRNA encoding the protein.
133. A pharmaceutical composition comprising the antisense oligomer of any of the compositions of claims 77 to 132, and an excipient.
134. A method of treating a subject in need thereof by administering the pharmaceutical composition of claim 133 by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.
135. A pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of a deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript, wherein the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript comprises a retained intron, wherein the antisense oligomer induces splicing out of the retained intron from the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript; and a pharmaceutical acceptable excipient.
136. The pharmaceutical composition of claim 135, wherein the deficient CTNS, PAX2, CYP24A1 or PPARD mRNA transcript is a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript.
137. The pharmaceutical composition of claim 135 or 136, wherein the targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript is in the retained intron within the region +500 relative to the 5' splice site of the retained intron to -500 relative to the 3' spliced site of the retained intron.
138. The pharmaceutical composition of claim 135 or 136, wherein the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript is encoded by a genetic sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 1-4.
139. The pharmaceutical composition of claim 135 or 136, wherein the CTNS, PAX2, CYP24A1 or PPARD

RIC pre-mRNA transcript comprises a sequence with at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NOs: 5-19.

140. The pharmaceutical composition of claim 135, wherein the antisense oligomer comprises a backbone modification comprising a phosphorothioate linkage or a phosphorodiamidate linkage.

141. The pharmaceutical composition of claim 135, wherein the antisense oligomer is an antisense oligonucleotide.

142. The pharmaceutical composition of claim 135, wherein the antisense oligomer comprises a phosphorodiamidate morpholino, a locked nucleic acid, a peptide nucleic acid, a 2'-O-methyl, a 2'-Fluoro, or a 2'-O-methoxyethyl moiety.

143. The pharmaceutical composition of claim 135, wherein the antisense oligomer comprises at least one modified sugar moiety.

144. The pharmaceutical composition of claim 135, wherein the antisense oligomer comprises from 8 to 50 nucleobases, 8 to 40 nucleobases, 8 to 35 nucleobases, 8 to 30 nucleobases, 8 to 25 nucleobases, 8 to 20 nucleobases, 8 to 15 nucleobases, 9 to 50 nucleobases, 9 to 40 nucleobases, 9 to 35 nucleobases, 9 to 30 nucleobases, 9 to 25 nucleobases, 9 to 20 nucleobases, 9 to 15 nucleobases, 10 to 50 nucleobases, 10 to 40 nucleobases, 10 to 35 nucleobases, 10 to 30 nucleobases, 10 to 25 nucleobases, 10 to 20 nucleobases, 10 to 15 nucleobases, 11 to 50 nucleobases, 11 to 40 nucleobases, 11 to 35 nucleobases, 11 to 30 nucleobases, 11 to 25 nucleobases, 11 to 20 nucleobases, 11 to 15 nucleobases, 12 to 50 nucleobases, 12 to 40 nucleobases, 12 to 35 nucleobases, 12 to 30 nucleobases, 12 to 25 nucleobases, 12 to 20 nucleobases, or 12 to 15 nucleobases.

145. The pharmaceutical composition of claim 135 or 136, wherein the antisense oligomer is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or is 100% complementary to a targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript.

146. The pharmaceutical composition of claim 135 or 136, wherein the targeted portion of the CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript is within a sequence selected from SEQ ID NOs: 10034-10748.

147. The pharmaceutical composition of claim 135, wherein the antisense oligomer comprises a nucleotide sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 20-10733.

148. The pharmaceutical composition of claim 135, wherein the antisense oligomer comprises a nucleotide sequence selected from SEQ ID NOs: 20-10733.

149. The pharmaceutical composition of any one of the claims 135-148, wherein the pharmaceutical composition is formulated for intrathecal injection, intracerebroventricular injection, intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection.

150. A method of inducing processing of a CTNS, PAX2, CYP24A1 or PPARD mRNA transcript to facilitate removal of a retained intron to produce a fully processed CTNS, PAX2, CYP24A1 or PPARD

mRNA transcript that encodes a functional form of a CTNS, PAX2, CYP24A1 or PPARD protein, the method comprising:

(a) contacting an antisense oligomer to a target cell of a subject;

(b) hybridizing the antisense oligomer to the CTNS, PAX2, CYP24A1 or PPARD mRNA transcript, wherein the CTNS, PAX2, CYP24A1 or PPARD mRNA transcript is capable of encoding the functional form of a CTNS, PAX2, CYP24A1 or PPARD protein and comprises at least one retained intron;

(c) removing the at least one retained intron from the CTNS, PAX2, CYP24A1 or PPARD mRNA transcript to produce the fully processed CTNS, PAX2, CYP24A1 or PPARD mRNA transcript that encodes the functional form of CTNS, PAX2, CYP24A1 or PPARD protein; and

(d) translating the functional form of CTNS, PAX2, CYP24A1 or PPARD protein from the fully processed CTNS, PAX2, CYP24A1 or PPARD mRNA transcript.

151. The method of claim 150, wherein the retained intron is an entire retained intron.

152. The method of claim 150 or 151, wherein the CTNS, PAX2, CYP24A1 or PPARD mRNA transcript is a CTNS, PAX2, CYP24A1 or PPARD RIC pre-mRNA transcript.

153. A method of treating a subject having a condition caused by a deficient amount or activity of CTNS, PAX2, CYP24A1 or PPARD protein comprising administering to the subject an antisense oligomer comprising a nucleotide sequence with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 20-10733.

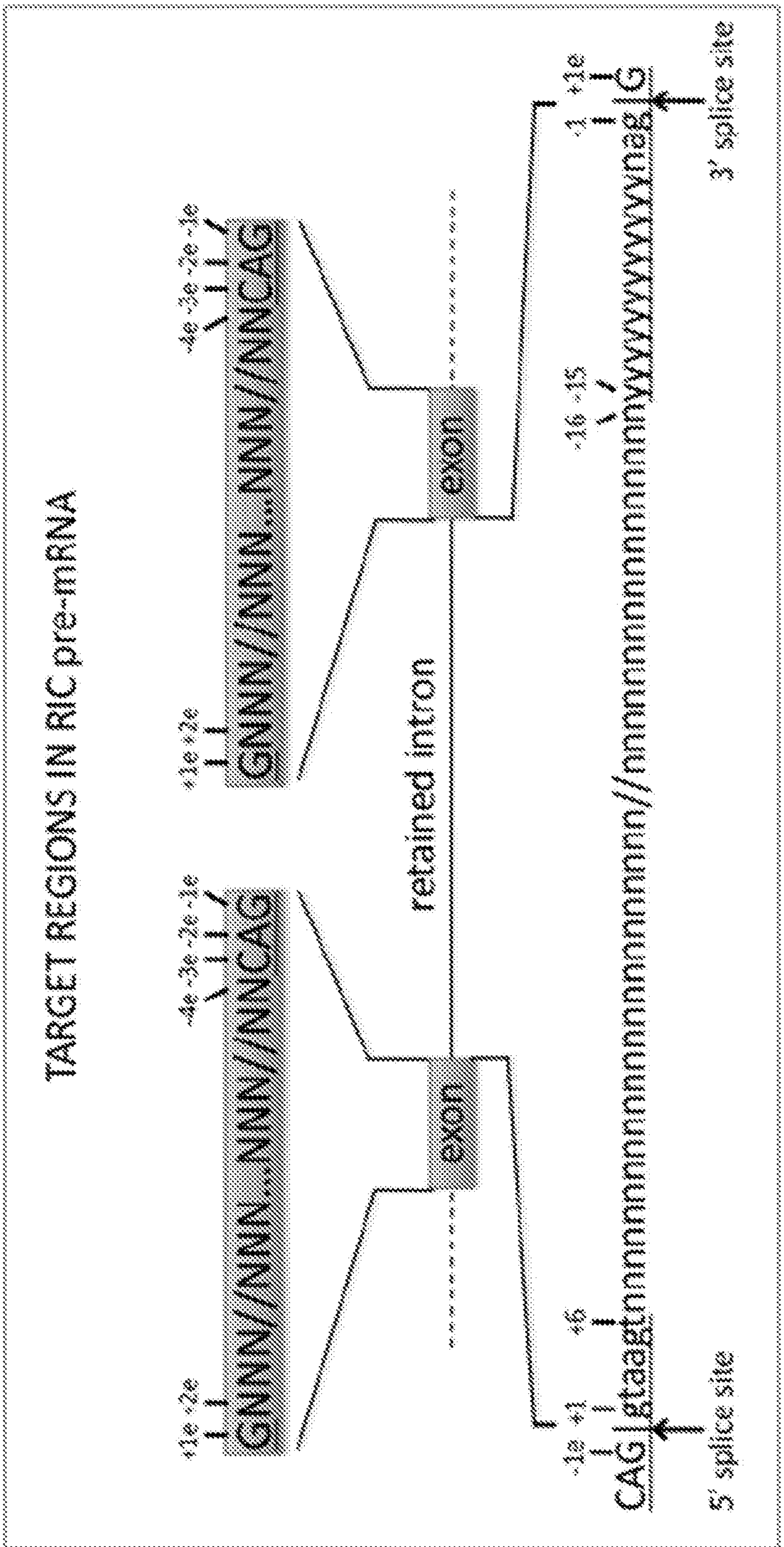

TARGET REGIONS IN RIC pre-mRNA
GNNN/NNN...NNN/NNCAG
GNNN/NNN...NNN/NNCAG
exon
retained intron
exon
CAG|gtaagtnnnnnnnnnnnnnnnnn//nnnnnnnnnnnnnnnnyyyyyyyyyyyynag|G
5' splice site
3' splice site

FIG. 1

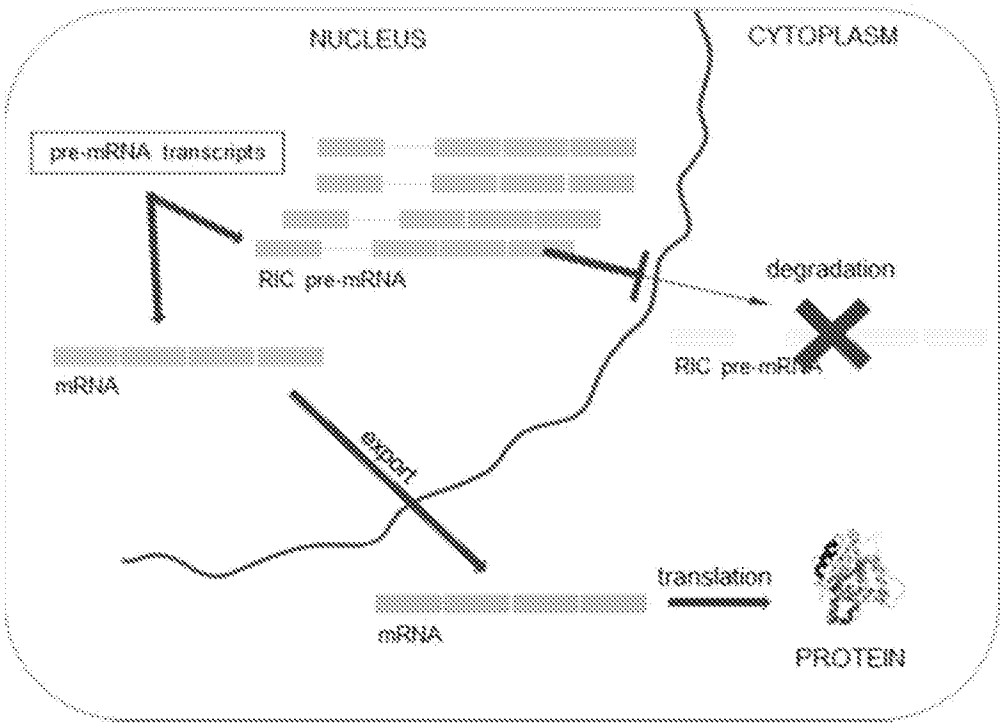

NUCLEUS
CYTOPLASM
pre-mRNA transcripts
RIC pre-mRNA
degradation
RIC pre-mRNA
mRNA
export
translation
mRNA
PROTEIN

FIG. 2A

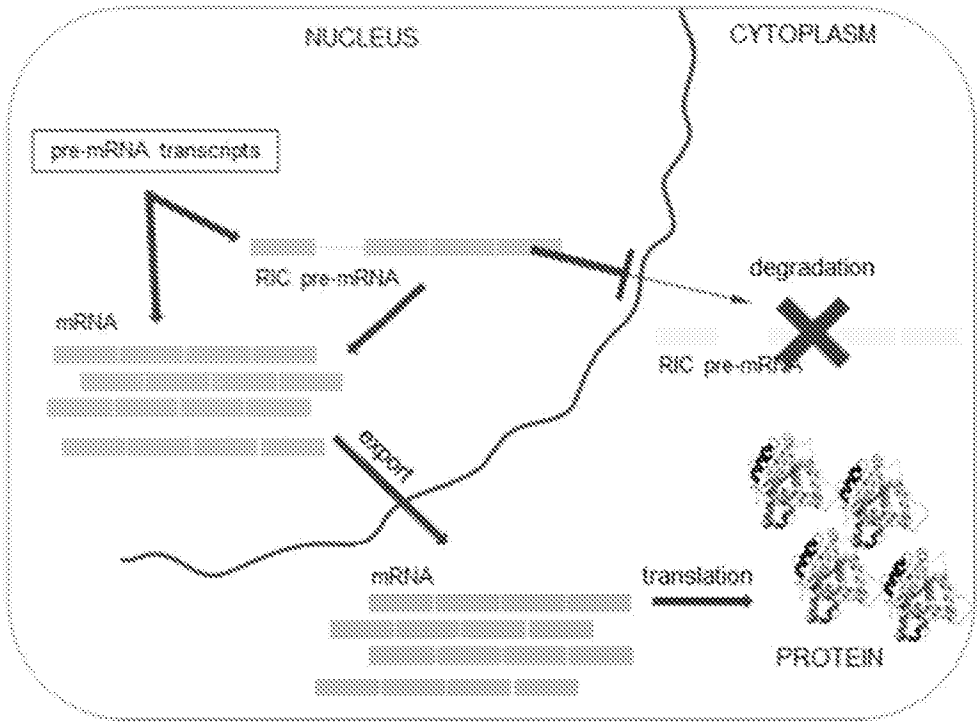

NUCLEUS
CYTOPLASM
pre-mRNA transcripts
RIC pre-mRNA
degradation
mRNA
RIC pre-mRNA
export
mRNA
translation
PROTEIN

FIG. 2B

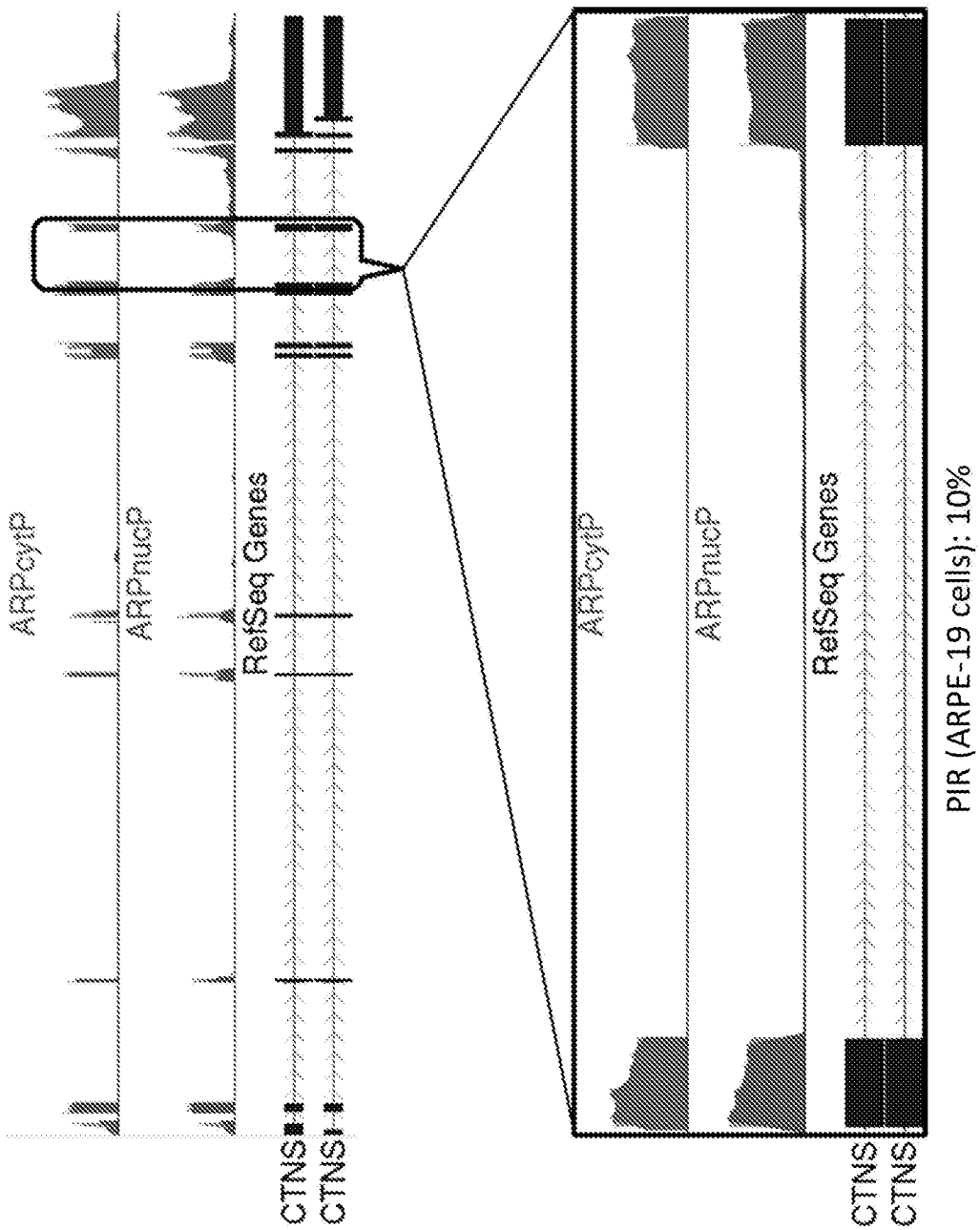

ARPcytP
ARPnucP
RefSeq Genes
CTNS
CTNS
ARPcytP
ARPnucP
RefSeq Genes
CTNS
CTNS
PIR (ARPE-19 cells): 10%

FIG. 3A

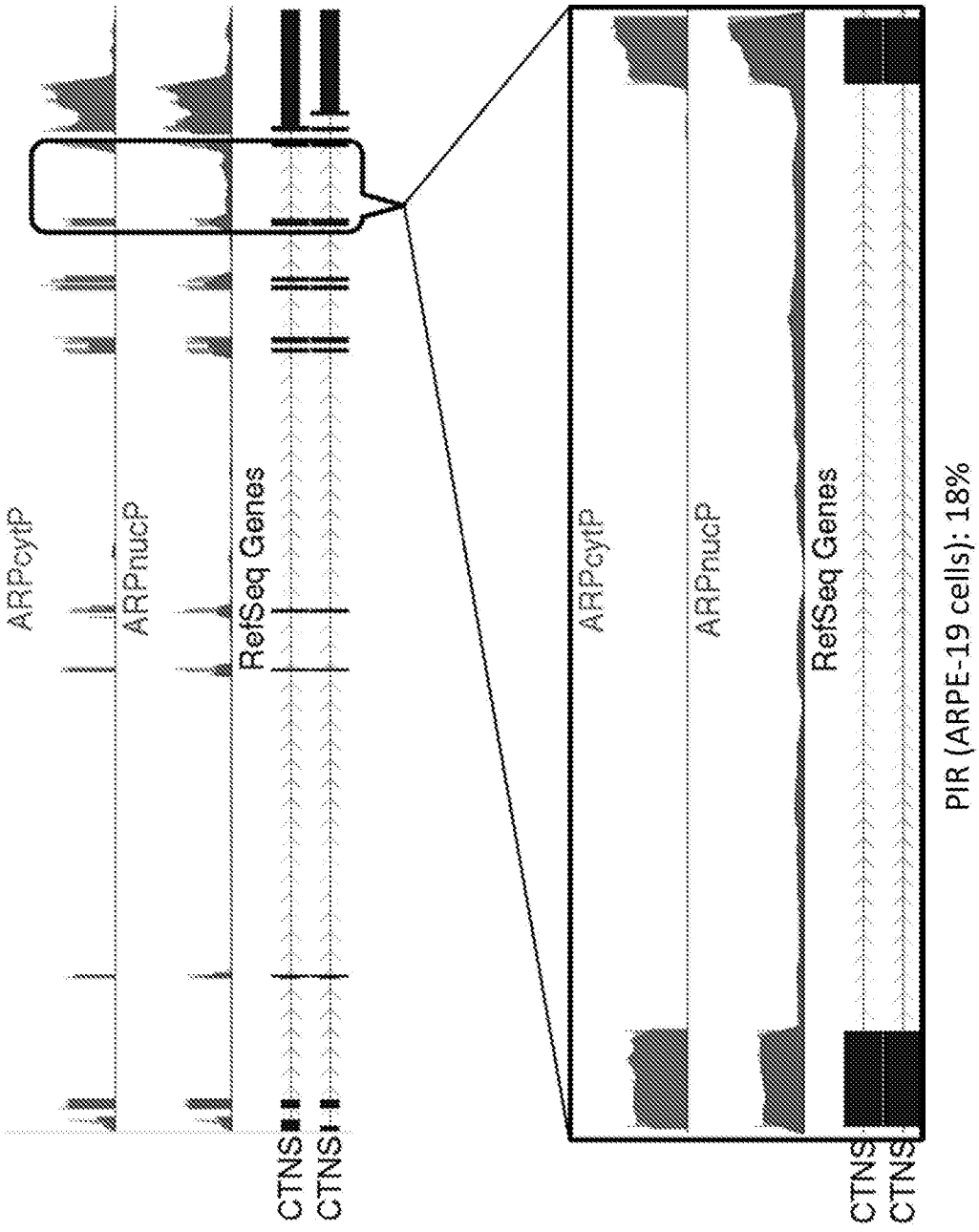

ARPcytP
ARPnucP
RefSeq Genes
CTNS
CTNS
ARPcytP
ARPnucP
RefSeq Genes
CTNS
CTNS
PIR (ARPE-19 cells): 18%

FIG. 3B

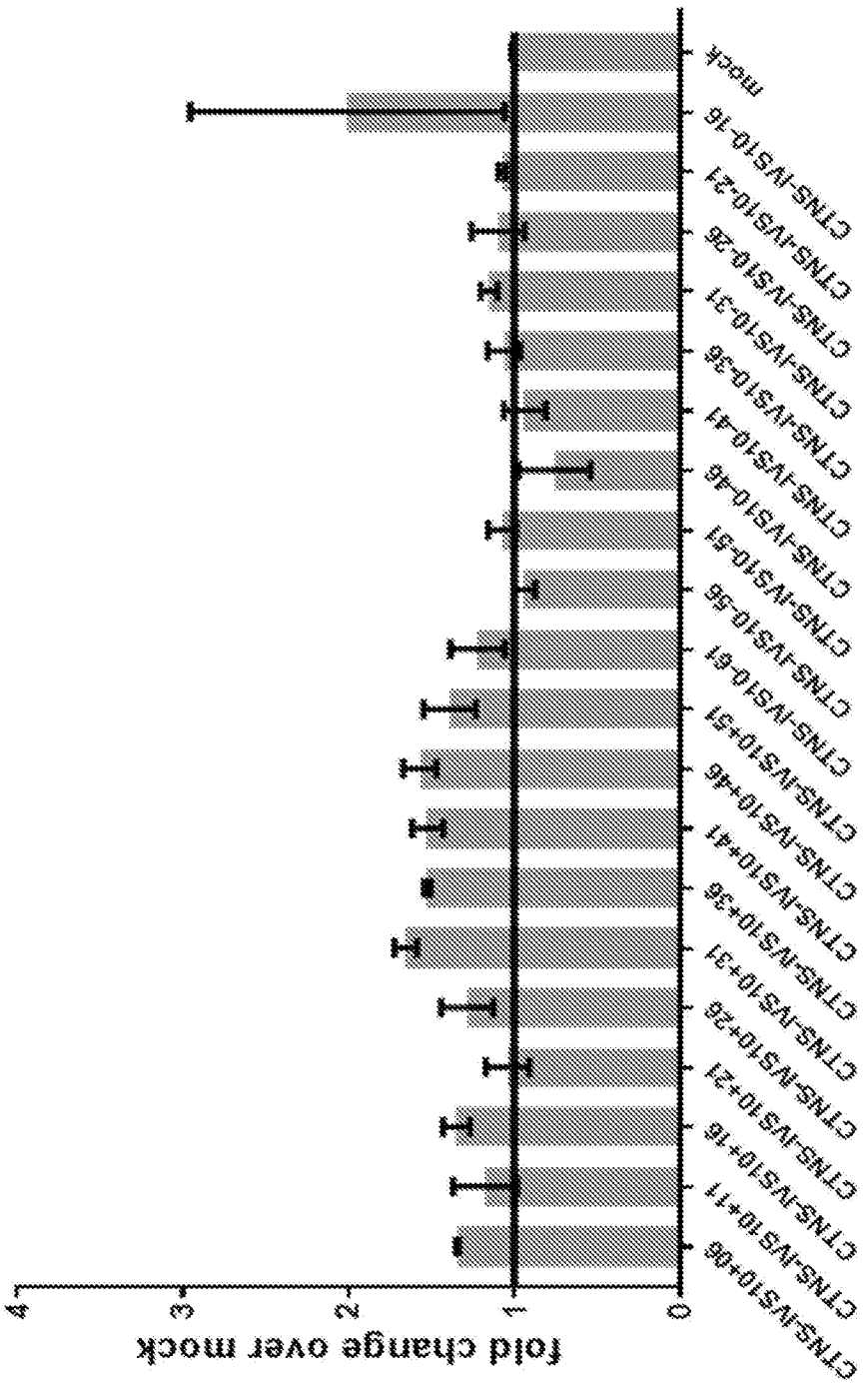

fold change over mock
0
1
2
3
4
mock
CTNS-IVS10-16
CTNS-IVS10-21
CTNS-IVS10-26
CTNS-IVS10-31
CTNS-IVS10-36
CTNS-IVS10-41
CTNS-IVS10-46
CTNS-IVS10-51
CTNS-IVS10-56
CTNS-IVS10-61
CTNS-IVS10+51
CTNS-IVS10+46
CTNS-IVS10+41
CTNS-IVS10+36
CTNS-IVS10+31
CTNS-IVS10+26
CTNS-IVS10+21
CTNS-IVS10+16
CTNS-IVS10+11
CTNS-IVS10+06

FIG. 3C

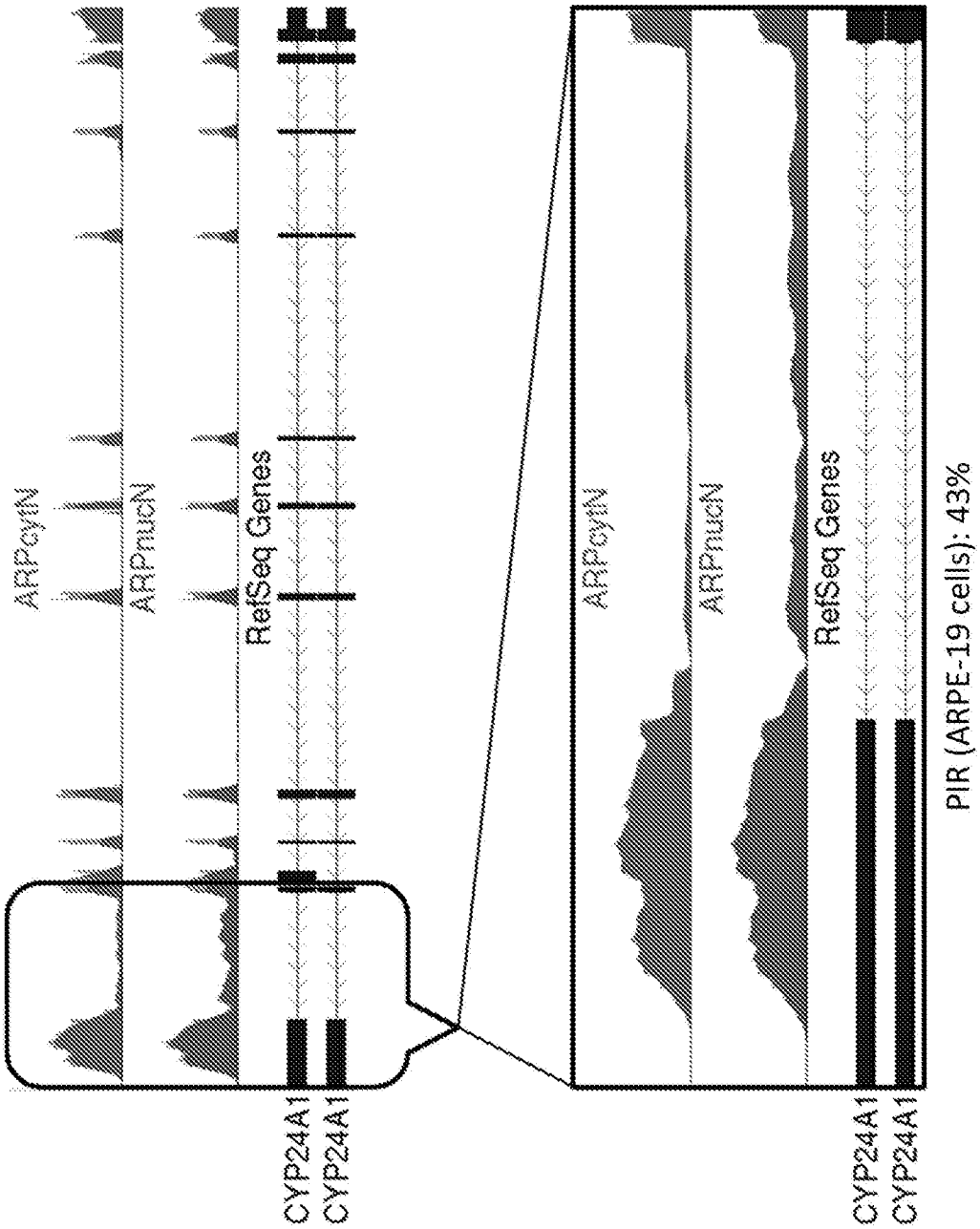

ARPcytN
ARPnucN
RefSeq Genes
CYP24A1
CYP24A1
ARPcytN
ARPnucN
RefSeq Genes
CYP24A1
CYP24A1
PIR (ARPE-19 cells): 43%

FIG. 4A

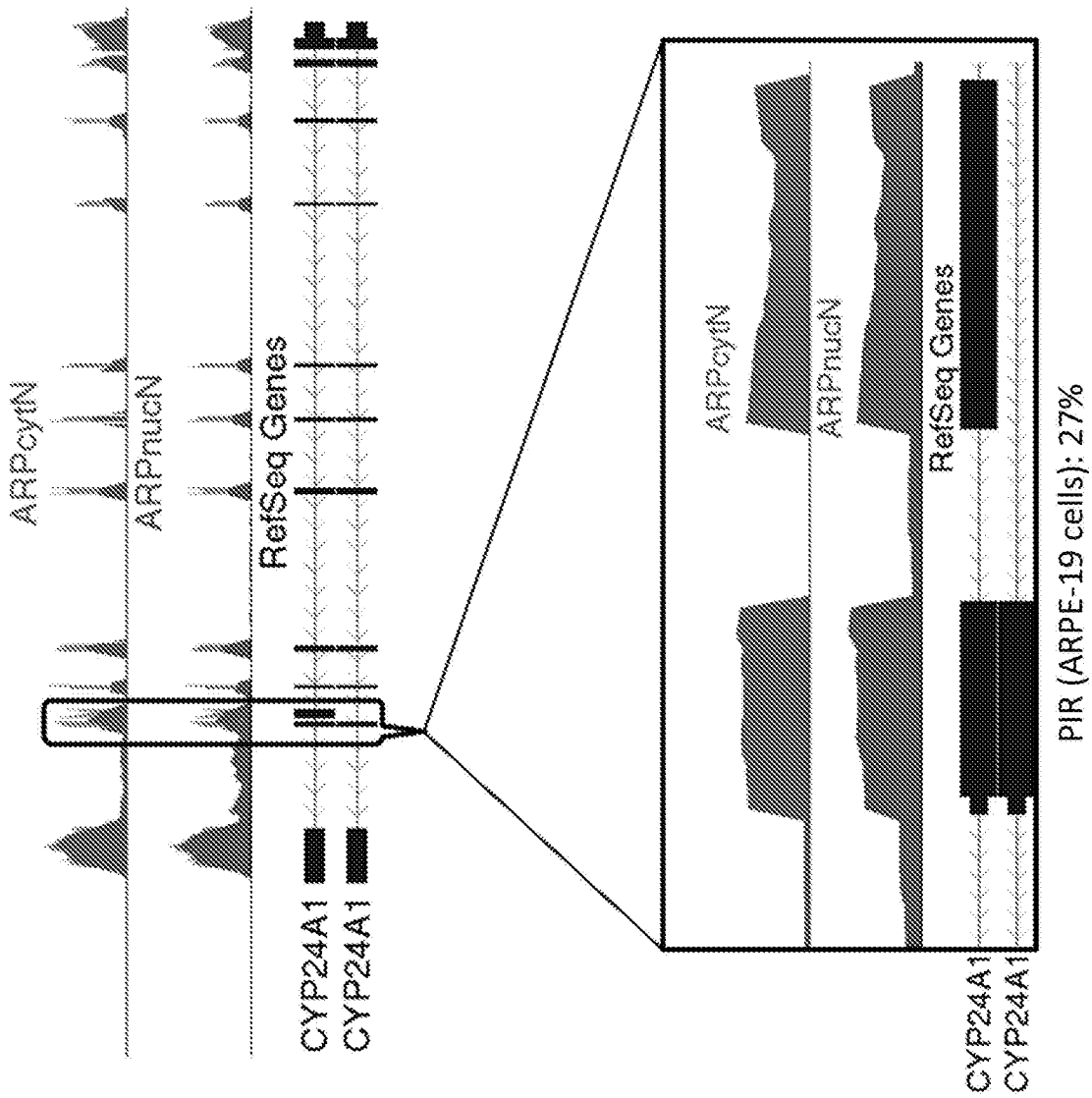

ARPcytN
ARPnucN
RefSeq Genes
CYP24A1
CYP24A1
ARPcytN
ARPnucN
RefSeq Genes
CYP24A1
CYP24A1
PIR (ARPE-19 cells): 27%

FIG. 4B

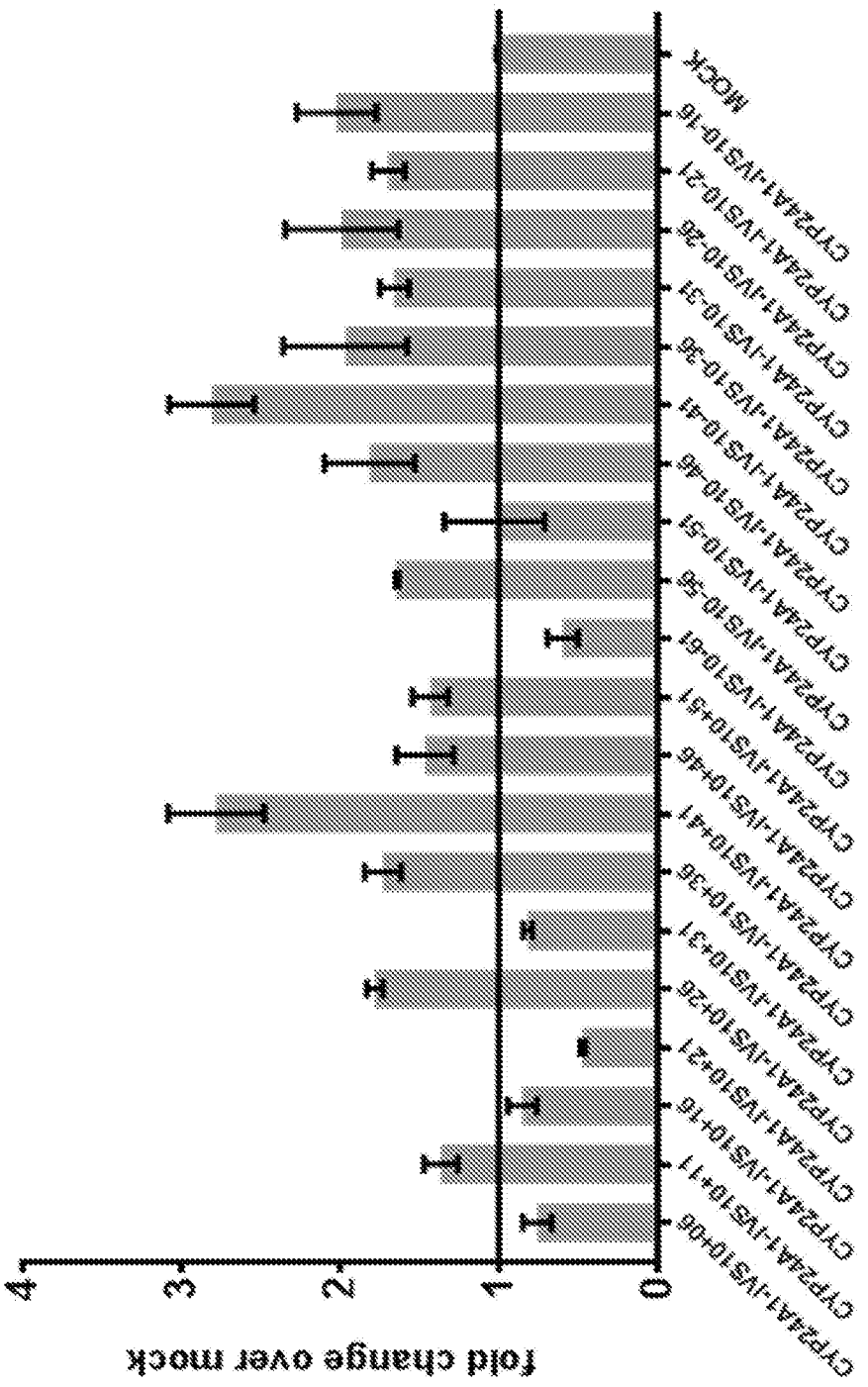

fold change over mock
0
1
2
3
4
CYP2AA1-JVS10-06
CYP2AA1-JVS10-11
CYP2AA1-JVS10-16
CYP2AA1-JVS10-21
CYP2AA1-JVS10-26
CYP2AA1-JVS10-31
CYP2AA1-JVS10-36
CYP2AA1-JVS10-41
CYP2AA1-JVS10-46
CYP2AA1-JVS10-51
CYP2AA1-JVS10-56
CYP2AA1-JVS10-61
CYP2AA1-JVS10-51
CYP2AA1-JVS10-46
CYP2AA1-JVS10-41
CYP2AA1-JVS10-36
CYP2AA1-JVS10-31
CYP2AA1-JVS10-26
CYP2AA1-JVS10-21
CYP2AA1-JVS10-16
CYP2AA1-JVS10-11
CYP2AA1-JVS10-06
MOCK

FIG. 4C

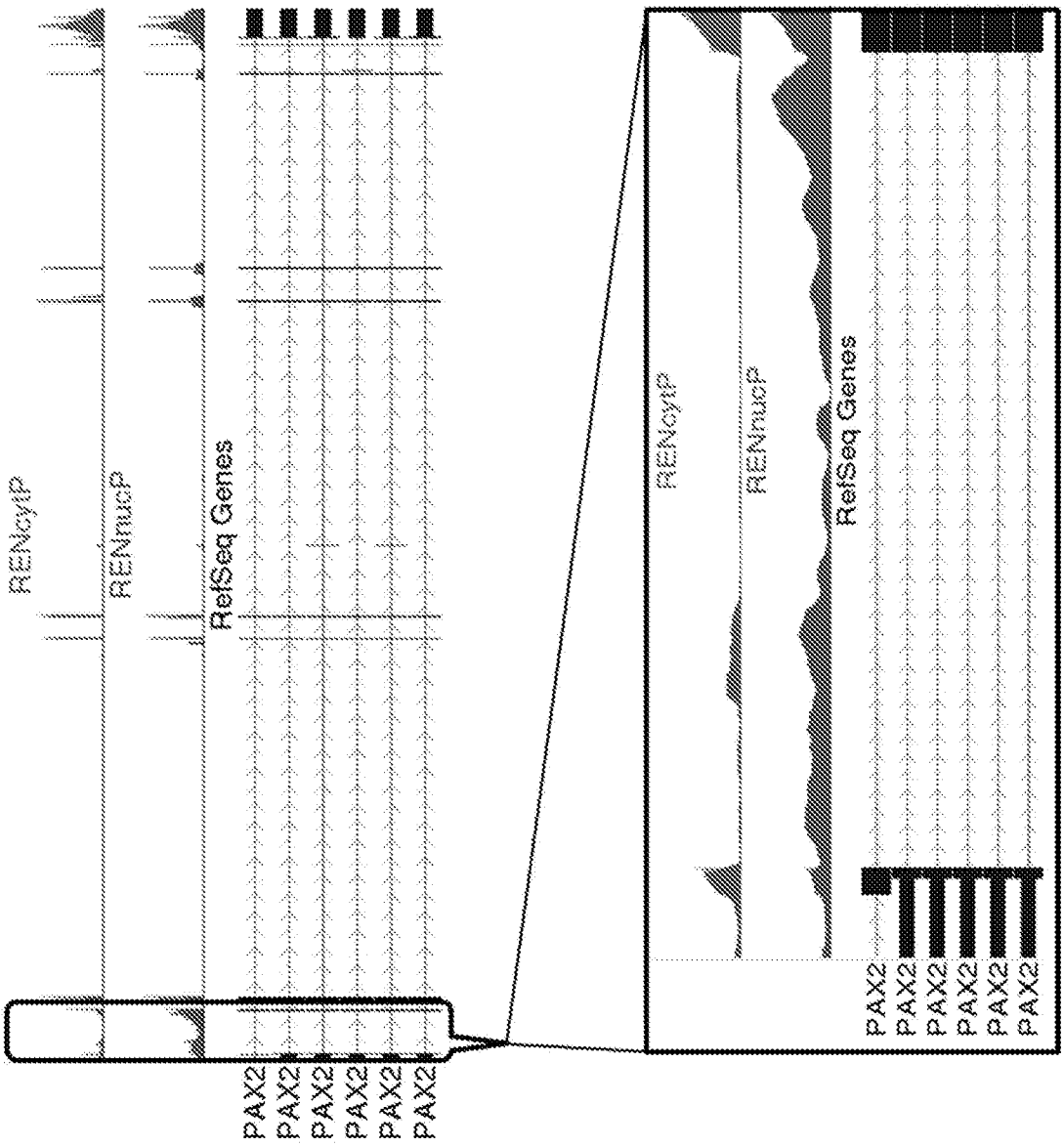
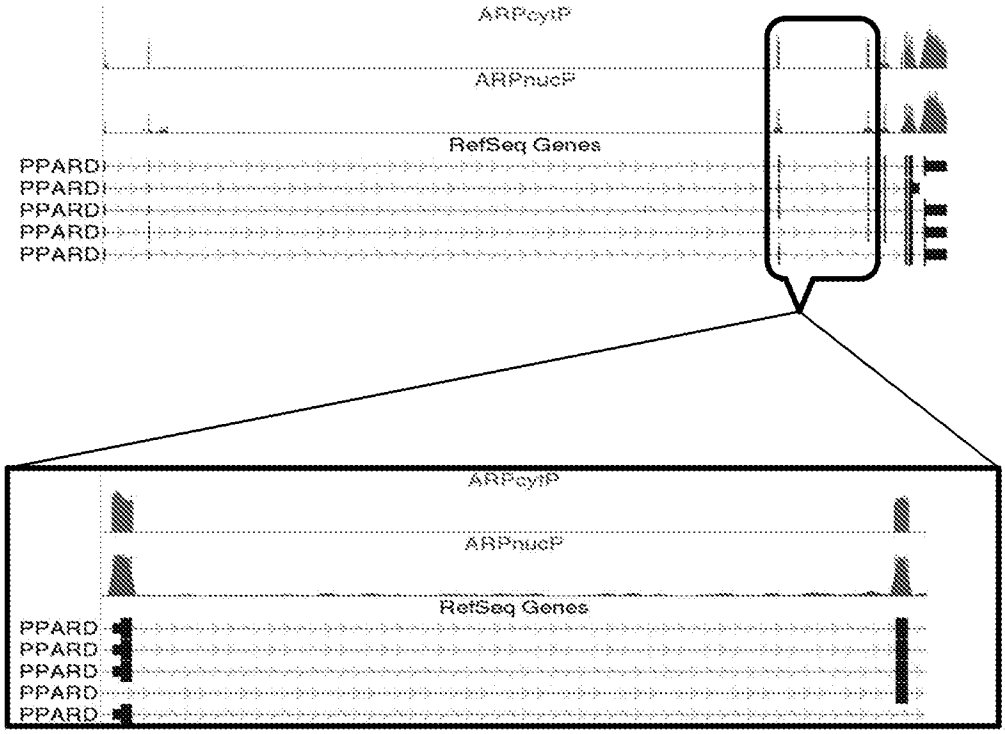

RENcytP
RENnucP
RefSeq Genes
PAX2
PAX2
PAX2
PAX2
PAX2
PAX2
RENcytP
RENnucP
RefSeq Genes
PAX2
PAX2
PAX2
PAX2
PAX2
PAX2

FIG. 5



PIR (ARPE-19 cells) 7%

FIG. 6A

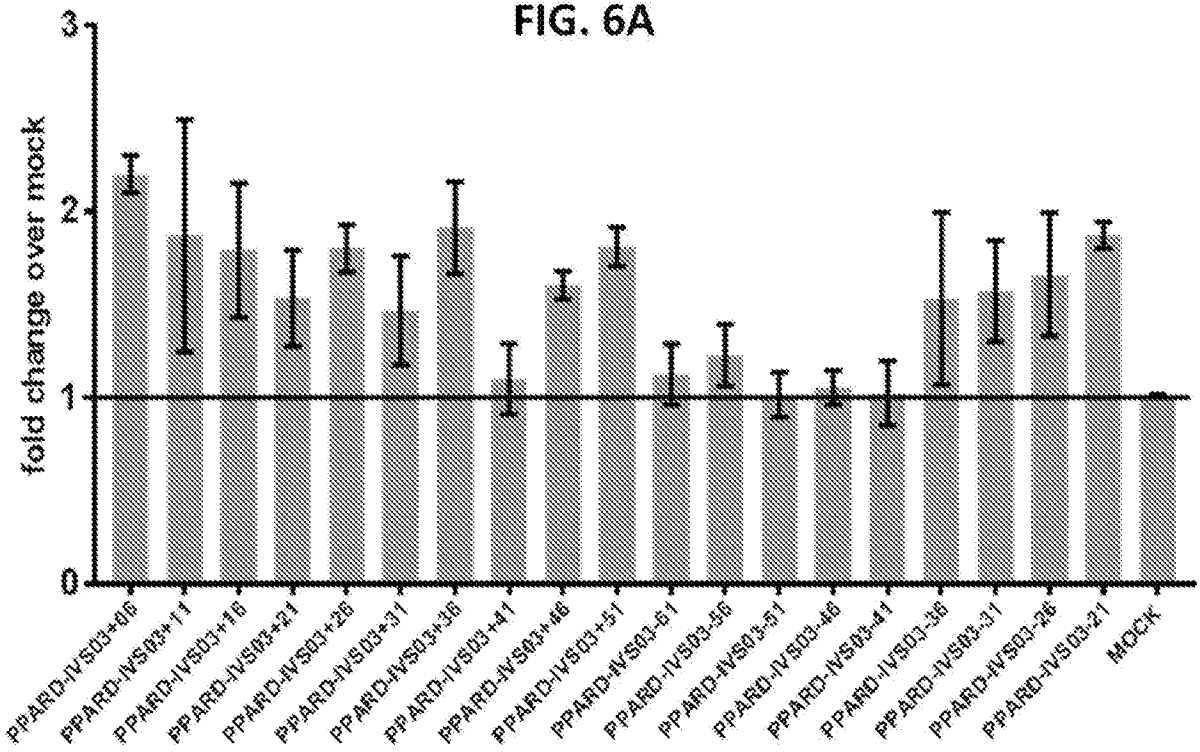


FIG. 6B

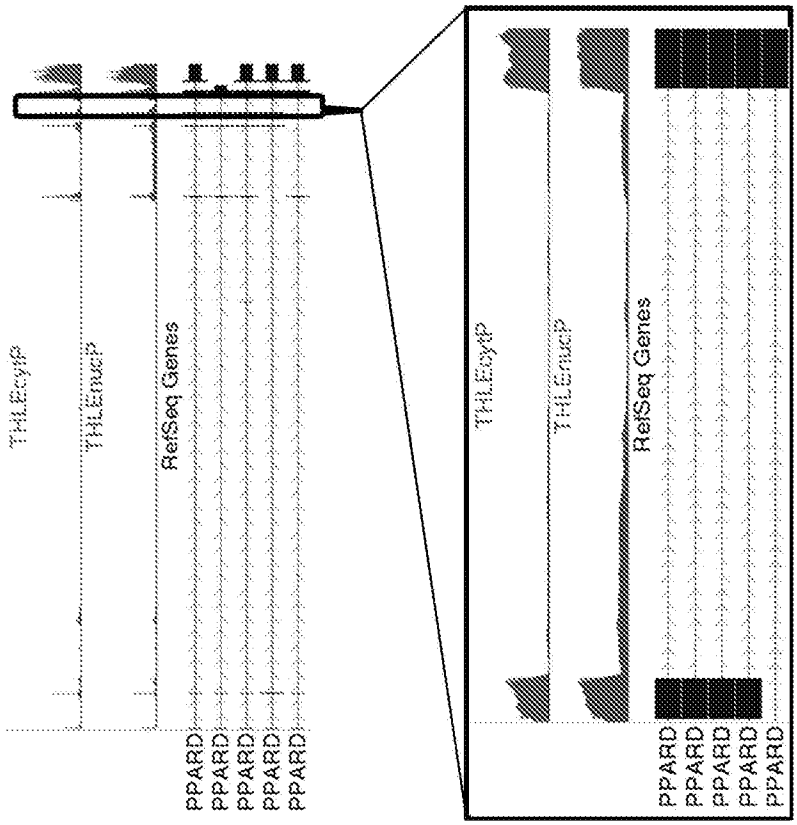

THLEcytP
THLEnucP
RefSeq Genes
PPARD
PPARD
PPARD
PPARD
PPARD
PIR (THLE cells) 12%

FIG. 6D

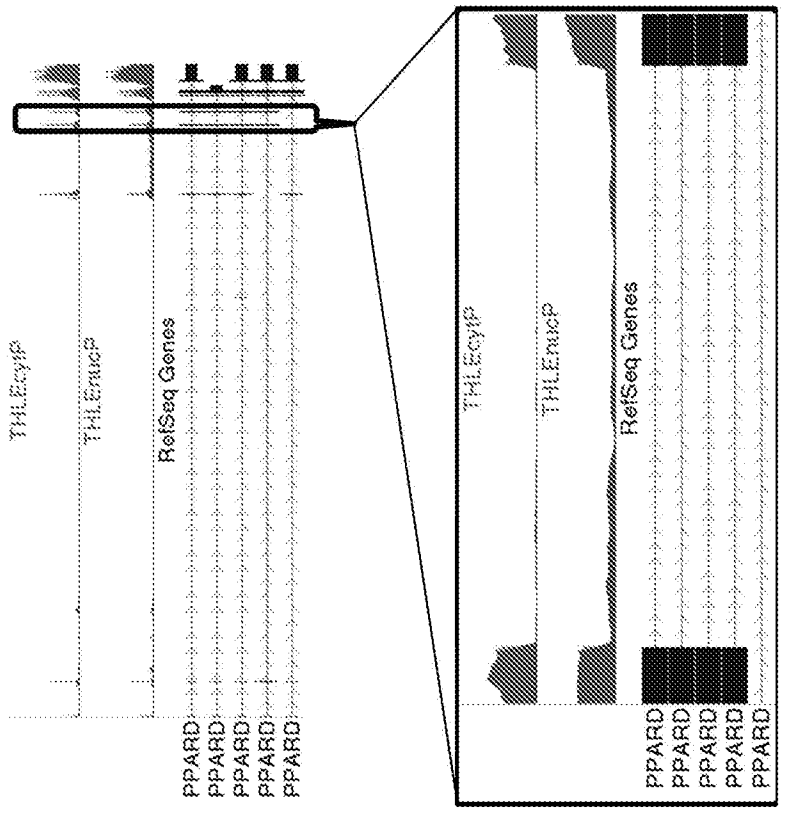
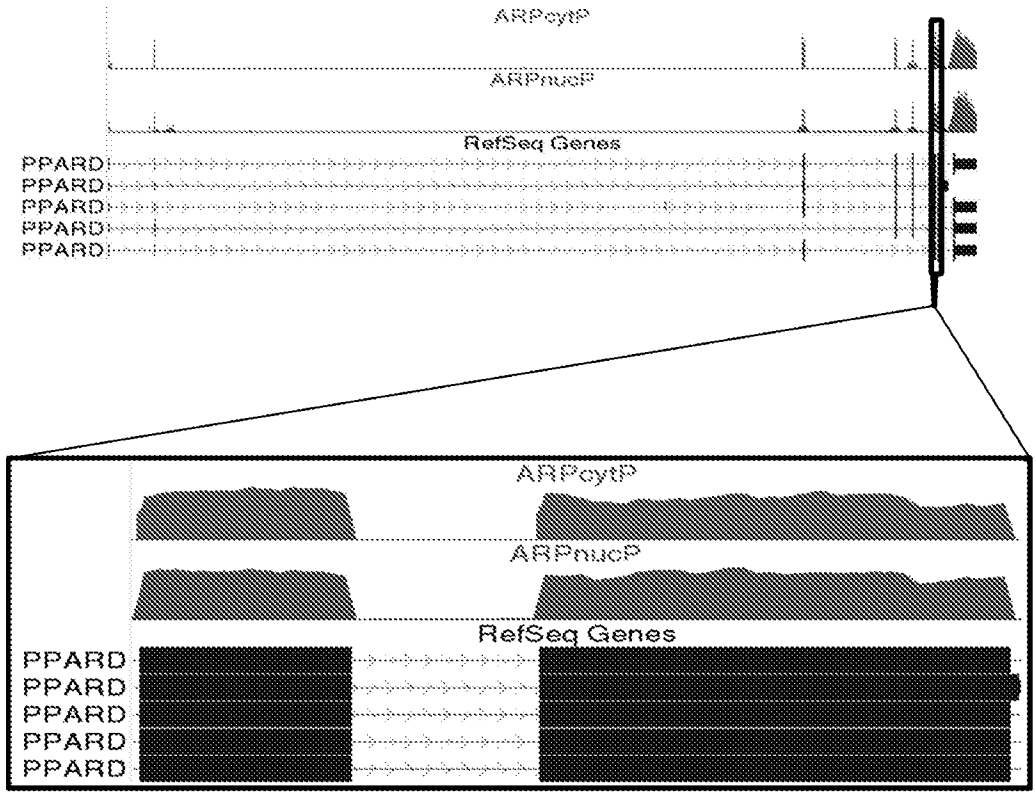

THLEcytP
THLEnucP
RefSeq Genes
PPARD
PPARD
PPARD
PPARD
PPARD
PIR (THLE cells) 18%

FIG. 6C



PIR (ARPE-19 cells) 7%

FIG. 6E

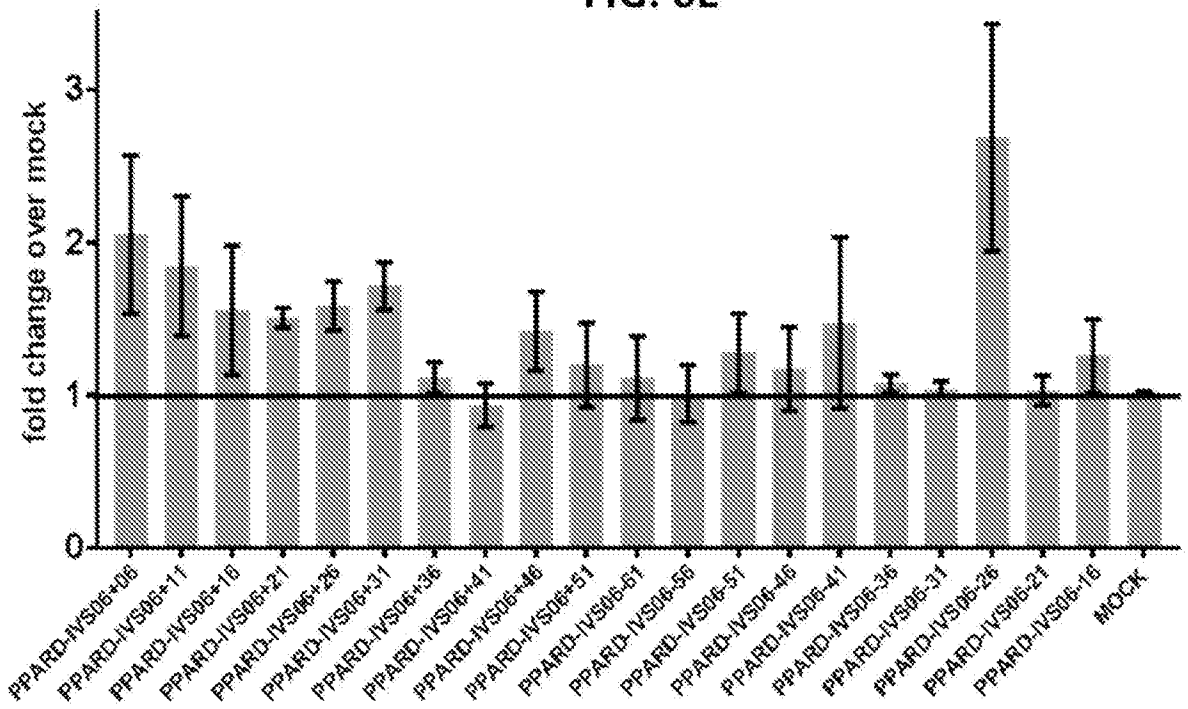


FIG. 6F

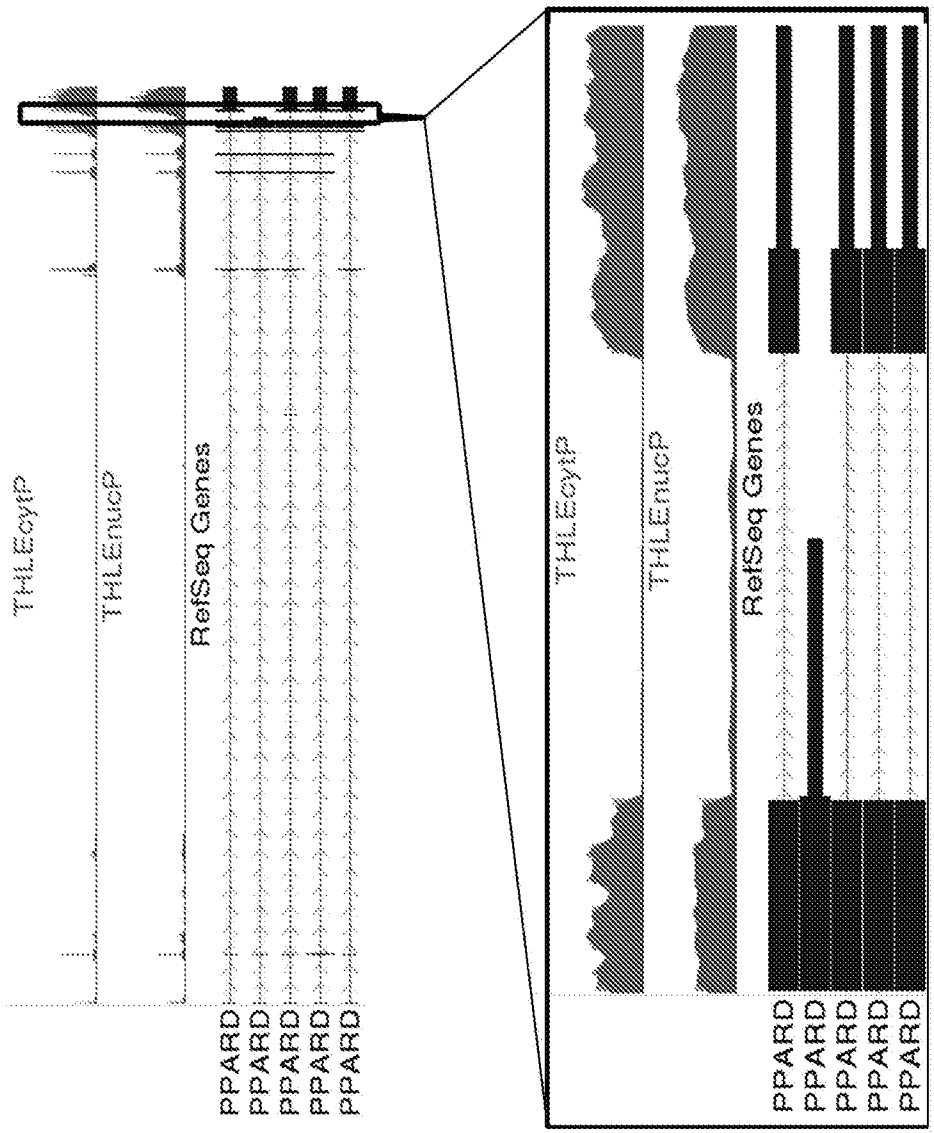

THLEcytP
THLEnucP
RefSeq Genes
PPARD
PPARD
PPARD
PPARD
PPARD
THLEcytP
THLEnucP
RefSeq Genes
PPARD
PPARD
PPARD
PPARD
PPARD
PIR N/A

FIG. 6G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/66576

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C12Q 1/68; C12N 15/11, 15/113, 15/52 (2017.01)
CPC - C12Q 1/6813, 1/6816, 1/6883, 1/6886; C12N 15/11, 15/111, 15/113, 15/52

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015/0337310 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 26 November, 2015; paragraphs [0010], [0012], [0016], [0022], [0025]-[0026], [0028], [0037], [0064]; figure 1B; claim 1	1-3, 4/1-2, 5/1-2, 6, 77-79, 80/77-79, 81, 82/80/77-79, 83/82/80/77-79, 84/82/80/77-79, 84/83/82/80/77-79, 135-136, 137/135-136, 138/135-136, 139/135-136, 140-144, 145/135-136, 146/135-136, 147-148, 150-151, 152/150-151,153
A	(KRALOVICOVA, J et al.) Optimal Antisense Target Reducing INS Intron 1 Retention is Adjacent to a Parallel G Quadruplex. Nucleic Acids Research. 17 June, 2014; Vol. 42, No. 12; pages 8161-8173; page 8162, column 1, paragraph 3; page 8163, column 1, paragraph 1; page 8165, column 1, paragraph 1; page 8171, column 1, paragraph 1; page 8171, column 2, paragraph 1; DOI: 10.1093/nar/gku507	1-3, 4/1-2, 5/1-2, 6, 77-79, 80/77-79, 81, 82/80/77-79, 83/82/80/77-79, 84/82/80/77-79, 84/83/82/80/77-79
A	(LEHIR, H et al.) 5'-End RET Splicing: Absence of Variants in Normal Tissues and Intron Retention in Pheochromocytomas. Oncology. 16 August, 2002; Vol. 63, No. 1; pages 84-91; abstract; page 85, column 1, paragraph 1; page 86, column 2, paragraph 1; DOI: 65725	1-2, 4/1-2, 5/1-2, 79, 80/79, 81, 82/79, 83/82/80/79, 84/82/80/79, 84/83/82/80/79

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 April 2017 (12.04.2017)	04 MAY 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/66576

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/66576

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-73, 75, 76, 85-134, 149
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-Please See Supplemental Page-*-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Groups I+, Claims 1-6, 74, 77-84, 135-148, 150-153, cytinosin (CTNS); SEQ ID NO: 20 (oligomer sequence); SEQ ID NO: 5 (target pre-mRNA sequence), SEQ ID NO: 10734 (target pre-mRNA sub-sequence), and SEQ ID NO: 1 (target pre-mRNA encoding sequence);

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/66576

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	(CORALLINI, S et al.) Transcriptional and Posttranscriptional Regulation of the CTNS Gene. Pediatric Research. August, 2011; Vol. 70, No. 2; pages 130-135; abstract; page 133, column 2, paragraph 2; DOI: 10.1203/PDR.0b013e3182200187	3, 6, 77-78, 80/77-78, 82/80/77-78, 83/82/80/77-78, 84/82/80/77-78, 84/83/82/80/77-78, 135-136, 137/135-136, 138/135-136, 139/135-136, 140-144, 145/135-136, 146/135-136, 147-148, 150-151, 152/150-151
A	(LUO, G et al.) Palmitic Acid Suppresses Apolipoprotein M Gene Expression via the Pathway of PPARb/d in HepG2 Cells. Biochemical and Biophysical Research Communications. 04 February, 2014; Vol. 445, No. 1; pages 203-207; page 204, column 1, paragraphs 1-2; Genbank supplement pages 1-5; DOI: 10.1016/j.bbrc.2014.01.170	74
A	US 2006/0134670 A1 (PIU, F) 22 June, 2006; paragraph [0046]	74, 153
A	US 2014/0235605 A1 (CELERA CORPORATION) 21 August, 2014; paragraph [0050]	74, 153
A	US 2004/0063129 A1 (GAARDE, W et al.) 01 April, 2004; paragraphs [0013]-[0014], [0018]	135-136, 137/135-136, 138/135-136, 139/135-136, 140-144, 145/135-136, 146/135-136, 147-148, 150-151, 152/150-151, 153
P-X	US 2016/0298121 A1 (COLD SPRING HARBOR LABORATORY) 13 October, 2016; whole document	1-3, 4/1-2, 5/1-2, 6, 74, 77-79, 80/77-79, 81, 82/80/77-79, 83/82/80/77-79, 84/82/80/77-79, 84/83/82/80/77-79, 135-136, 137/135-136, 138/135-136, 139/135-136, 140-144, 145/135-136, 146/135-136, 147-148, 150-151, 152/150-151, 153

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/66576

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-6, 74, 77-84, 135-148, 150-153, cytinosin (CTNS), and SEQ ID NOs: 1, 5, 20 and 10734 are directed toward a method of treating kidney disease by increasing expression and processing of an mRNA transcript of a target gene, wherein the pre-mRNA has a retained intron; compositions and oligomers associated with the methods.

The methods, compositions and oligomers will be searched to the extent the oligomer encompasses SEQ ID NO: 20 (first exemplary oligomer sequence), and targets a transcript encompassing SEQ ID NO: 5 (first exemplary target pre-mRNA sequence), encoded by SEQ ID NO: 1 (first exemplary target pre-mRNA encoding sequence); and wherein the targeted sequence further includes a targeted sub-sequence encompassing SEQ ID NO: 10734 (first exemplary target pre-mRNA sub-sequence). Applicant is invited to elect additional target gene(s) and/or associated oligomer(s), with specified SEQ ID NO: for each, and, where applicable, associated target pre-mRNA, target pre-mRNA sub-sequence and target pre-mRNA encoding sequence(s) associated with the elected oligomer sequence(s), to be searched. Additional target gene(s), and/or oligomer(s) and, where applicable, associated pre-mRNA and pre-mRNA encoding sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2, 3 (in-part), 4 (in-part), 5, 6, 74 (in-part), 77-79, 80 (in-part), 81 82 (in-part), 83 (in-part), 84 (in-part), 135 (in-part), 136 (in-part), 137 (in-part), 138 (in-part), 139 (in-part), 140 (in-part), 141 (in-part), 142 (in-part), 143 (in-part), 144 (in-part), 145 (in-part), 146 (in-part), 147 (in-part), 148 (in-part), 150 (in-part), 151 (in-part), 152 (in-part) and 153 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 20 (oligomer sequence); SEQ ID NO: 5 (target pre-mRNA sequence), SEQ ID NO: 10734 (target pre-mRNA sub-sequence), and SEQ ID NO: 1 (target pre-mRNA encoding sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an oligomer encompassing SEQ ID NO: 21 (first exemplary elected oligomer sequence).

No technical features are shared between the target genes and/or oligomer sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a method of treating a kidney disease in a subject in need thereof by increasing the expression of a target protein or functional RNA by cells of the subject, wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site, and wherein the RIC pre-mRNA encodes the target protein or functional RNA, the method comprising contacting the cells of the subject with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein or functional RNA, whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA, thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject; a method of increasing expression of a target protein by cells having a retained-intron-containing pre-mRNA (RIC pre-mRNA), the method comprising contacting the cells with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein; an antisense oligomer comprising a sequence; a method of treating a subject having a condition caused by a deficient amount or activity of a protein, comprising administering to the subject an antisense oligomer comprising a nucleotide sequence; a composition comprising an antisense oligomer for use in a method of increasing expression of a target protein or a functional RNA by cells to treat a kidney disease in a subject in need thereof associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject, wherein the antisense oligomer enhances constitutive splicing of a retained intron-containing pre-mRNA (RIC pre-mRNA) encoding the target protein or the functional RNA, wherein the target protein is: (a) the deficient protein; or (b) a compensating protein which functionally augments or replaces the deficient protein or in the subject; and wherein the functional RNA is: (a) the deficient RNA; or (b) a compensating functional RNA which functionally augments or replaces the deficient functional RNA in the subject; a composition comprising an antisense oligomer for use in a method of treating a condition associated with a target protein in a subject in need thereof, the method comprising the step of increasing expression of the target protein by cells of the subject, a pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of a deficient mRNA transcript, wherein the deficient mRNA transcript comprises a retained intron, wherein the antisense oligomer induces splicing out of the retained intron from the deficient mRNA transcript; and a pharmaceutical acceptable excipient; a method of inducing processing of a mRNA transcript to facilitate removal of a retained intron to produce a fully processed mRNA transcript that encodes a functional form of a protein, the method comprising: (a) contacting an antisense oligomer to a target cell of a subject; (b) hybridizing the antisense oligomer to the mRNA transcript, wherein the mRNA transcript is capable of encoding the functional form of a protein and comprises at least one retained intron; (c) removing the at least one retained intron from the mRNA transcript to produce the fully processed mRNA transcript that encodes the functional form of the protein; and (d) translating the functional form of the protein from the fully processed mRNA transcript.

However, these shared technical features are previously disclosed by US 2015/0337310 A1 to The Regents of the University of California (hereinafter 'California') in view of the article 'Optimal antisense target reducing INS intron 1 retention is adjacent to a parallel G quadruplex' by Kralovicova et al. (hereinafter 'Kralovicova') and the article "5'-End RET Splicing: Absence of Variants in Normal Tissues and Intron Retention in Pheochromocytomas" by Le Hir et al. (hereinafter 'Le Hir').

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

California discloses a method of treating a kidney disease (a method of treating cancer, including kidney cancer (a method of treating a kidney disease); abstract, paragraph [0025]) in a subject in need thereof (in a subject in need thereof; abstract, paragraph [0072]) by increasing the expression of a target protein or functional RNA by cells of the subject (by increasing the expression of a target protein or functional RNA by cells of the subject; abstract), wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA) (wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA); paragraph [0005]), and wherein the RIC pre-mRNA encodes the target protein or functional RNA (wherein the RIC pre-mRNA encodes the target protein or functional RNA; paragraphs [0005], [0010]), the method comprising contacting the cells of the subject with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein or functional RNA (the method comprising contacting the cells of the subject with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-mRNA encoding the target protein or functional RNA; paragraphs [0010], [0015]), thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject (thereby increasing the level of mRNA encoding the target protein or functional RNA, and increasing the expression of the target protein or functional RNA in the cells of the subject; paragraph [0010]); a method of increasing expression of a target protein by cells (a method of increasing expression of a target protein by cells; abstract, paragraph [0010]) having a retained-intron-containing pre-rnRNA (RIC pre-mRNA) (having a retained-intron-containing pre-rnRNA (RIC pre-mRNA); paragraph [0005]), the method comprising contacting the cells with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-rnRNA encoding the target protein (the method comprising contacting the cells with an antisense oligomer (ASO) complementary to a targeted portion of the RIC pre-rnRNA encoding the target protein; paragraphs [0010], [0015]); an antisense oligomer comprising a sequence (a complementary (an antisense) oligomer comprising a sequence; paragraphs [0015], [0016]); a method of treating a subject having a condition caused by a deficient amount or activity of a protein (a method of treating a subject having a condition caused by a deficient amount or activity of a protein; paragraph [0010]), comprising administering to the subject an antisense oligomer comprising a nucleotide sequence (comprising administering to the subject an antisense oligomer comprising a nucleotide sequence; paragraphs [0010], [0015], [0016]); a composition comprising an antisense oligomer (a composition comprising a complementary (an antisense) oligomer; paragraphs [0015], [0022]) for use in a method of increasing expression of a target protein or a functional RNA by cells to treat a kidney disease in a subject in need thereof (for use in a method of increasing expression of a target protein or a functional RNA by cells to treat cancer, including kidney cancer (a kidney disease) in a subject in need thereof; abstract, paragraphs [0010], [0025]) associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject (associated with a deficient protein or deficient functional RNA, wherein the deficient protein or deficient functional RNA is deficient in amount or activity in the subject; paragraph [0010]), wherein the antisense oligomer enhances constitutive splicing (wherein the antisense oligomer enhances constitutive splicing; paragraph [0010]) of a retained intron-containing pre-mRNA (RIC pre-mRNA) (of a retained intron-containing pre-mRNA (RIC pre-mRNA); paragraphs [0005], [0010]) encoding the target protein or the functional RNA, wherein the target protein is: (a) the deficient protein (encoding the target protein or the functional RNA, wherein the target protein is: (a) the deficient protein; paragraph [0010]); a composition comprising an antisense oligomer for use in a method (a composition comprising a complementary (an antisense) oligomer; paragraphs [0015], [0022]) of treating a condition associated with a target protein in a subject in need thereof (of treating a condition associated with a target protein in a subject in need thereof; paragraphs [0010], [0072]), the method comprising the step of increasing expression of the target protein by cells of the subject (the method comprising the step of increasing expression of the target protein by cells of the subject; paragraph [0010]), a pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of a deficient mRNA transcript (a pharmaceutical composition comprising: an antisense oligomer that hybridizes to a target sequence of a deficient mRNA transcript; paragraphs [0010], [0015], [0022]), wherein the deficient mRNA transcript comprises a retained intron (wherein the deficient mRNA transcript comprises a retained intron; paragraphs [0005], [0010]); and a pharmaceutically acceptable excipient (a pharmaceutically acceptable carrier (excipient); paragraph [0022]); a method of inducing processing of a mRNA transcript to produce a fully processed mRNA transcript that encodes a functional form of a protein (a method of inducing processing of a mRNA transcript to produce a fully processed mRNA transcript that encodes a functional form of a protein; paragraph [0010]), the method comprising: (a) contacting an antisense oligomer to a target cell of a subject (the method comprising: (a) contacting a complementary (an antisense) oligomer to a target cell of a subject; paragraphs [0010], [0015]); (b) hybridizing the antisense oligomer to the mRNA transcript (b) hybridizing the antisense oligomer to the mRNA transcript; paragraphs [0010], [0026]), wherein the mRNA transcript is capable of encoding the functional form of a protein (wherein the mRNA transcript is capable of encoding the functional form of a protein; paragraph [0010]) and comprises at least one retained intron (comprises at least one retained intron; paragraph [0005]); and (d) translating the functional form of the protein from the fully processed mRNA transcript (translating the functional form of the protein from the fully processed mRNA transcript; paragraph [0010]).

California does not disclose wherein the cells have a retained-intron-containing pre-mRNA (RIC pre-mRNA), the RIC pre-mRNA comprising a retained intron, an exon flanking the 5' splice site, an exon flanking the 3' splice site; whereby the retained intron is constitutively spliced from the RIC pre-mRNA encoding the target protein or functional RNA; wherein the antisense oligomer enhances or induces constitutive splicing of a deficient retained intron-containing pre-mRNA (RIC pre-mRNA) encoding the target protein or the functional RNA; to facilitate removal of a retained intron; removing the at least one retained intron from the mRNA transcript to produce the fully processed mRNA transcript that encodes the functional form of the protein. California further does not disclose specifically wherein the kidney cancer is associated with a retained intron.

Kralovicova discloses targeting a retained-intron-containing pre-mRNA with an antisense oligonucleotide (targeting a retained-intron-containing pre-mRNA with an antisense oligonucleotide; abstract) that reduces the number of transcripts that retain an intron (that reduces the number of transcripts that retain an intron; page 8162, first column, third paragraph) between two exons (between two exons; Figure 1).

Le Hir discloses wherein aberrant retention of intron 2 in a gene is associated with kidney tumors (aberrant retention of intron 2 in a gene is associated with kidney tumors; abstract).

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of California to have included the use of antisense oligonucleotides directed to enhance splicing, as disclosed by California, of mRNA containing a retained intron between two exons, as disclosed by Kralovicova, in order to enhance the production of the active protein by a cell, as disclosed by California, by removing the intervening retained intron, as disclosed by Kralovicova. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of California to have used the methods disclosed thereby to have enhanced the splicing of a transcript having a retained intron, as disclosed by California, wherein the presence of the retained intron was associated with kidney cancer, as disclosed by Le Hir, in order to enable removal of the retained intron to produce a functional protein, thereby treating the kidney cancer, based on the disclosure of California.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the California, Kralovicova and Le Hir references, unity of invention is lacking.