MODIFIED U1 SNRNAS

FIELD OF THE INVENTION

[0001] The present invention generally relates to the field of RNA splicing. In particular, the invention relates to modified U1 snRNAs configured to rescue exon skipping in a mutated *ABCA4* gene. The invention also relates to use of the modified U1 snRNAs as therapeutic candidates for *ABCA4* retinopathies.

BACKGROUND

[0002] Stargardt disease (STGD) is the most common inherited retinal disorder affecting the macula, whose degeneration leads to partial or complete blindness. In a significant number of cases, STGD is caused by loss-of-function and/or loss-of-expression of ABCA4 protein due to loss of exon, which is essential for maintaining a healthy retina.

[0003] As the coding sequence length of ABCA4 gene is beyond the cargo limit of AAVs, gene replacement therapy is not viable. Furthermore, a Phase 1/2 clinical trial using a lentiviral delivery vehicle to circumvent this issue has resulted in worsening condition of patients' eye vision. While cell-therapy strategies have been proposed to stop or mitigate the disease progression, none of them has been approved for therapy.

[0004] There is thus a need for new intervention strategies to rescue loss of exon caused by mis-splicing of a mutated ABCA4 gene that overcome the drawbacks of the prior art. Furthermore, other desirable features and characteristics will become apparent from the subsequent detailed description and the appended claims, taken in conjunction with the accompanying drawings and this background of the disclosure.

SUMMARY

[0005] In one aspect, the present invention provides a modified U1 small nuclear RNA (snRNA) configured to retain exon 40 in a mature mRNA transcript of a mutated ABCA4 gene, the mutated ABCA4 gene comprising a mutation that induces skipping of exon 40, wherein the mutation is located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40, wherein the modification comprises replacing a portion of a single-stranded nucleotide sequence of a 5' region of a wild-type U1 snRNA with a single-stranded binding nucleotide sequence capable of hybridizing to a target sequence present on a pre-mRNA transcript of the mutated ABCA4 gene, and wherein the target sequence is located in a region between 13 base pairs upstream and 15 base pairs downstream of the donor splice site of exon 40.

[0006] In one embodiment, the mutation that induces skipping of exon 40 is c.5714+5G>A.

[0007] In one embodiment, the region comprises the sequence of SEQ ID NO: 1.

[0008] In one embodiment, the single-stranded binding nucleotide sequence is between 11 and 21 nucleotides in length.

[0009] In one embodiment, the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 2 to 19.

[0010] In one embodiment, the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 7, 8 and 10.

[0011] In another aspect, the present invention provides a nucleic acid construct comprising a polynucleotide sequence encoding the modified U1 snRNA as described herein.

[0012] In one embodiment, the polynucleotide sequence encoding the modified U1 snRNA is operably linked to a RNU1-1 promoter and to a RNU1-1 terminator.

[0013] In one embodiment, the nucleic acid construct as described herein comprises the sequence of SEQ ID NO: 25.

[0014] In one embodiment, a portion of the polynucleotide sequence that encodes the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 26 to 43.

[0015] In one embodiment, a portion of the polynucleotide sequence that encodes the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 31, 32 and 34.

[0016] In one embodiment, the nucleic acid construct as described herein is contained in an expression vector.

[0017] In various embodiments, the expression vector is selected from the group consisting of a lentiviral vector and an adeno-associated viral vector.

[0018] In another embodiment, the nucleic acid construct as described herein is contained in a lipid nanoparticle.

[0019] In one aspect, there is provided a modified U1 snRNA as described herein or a nucleic acid construct as described herein for use in treating a ABCA4 retinopathy.

[0020] In one embodiment, the *ABCA4* retinopathy is Stargardt disease.

[0021] In another aspect, there is provided a use of a modified U1 snRNA as described herein and a nucleic acid construct as described herein in the manufacture of a medicament for treating a *ABCA4* retinopathy.

[0022] In one embodiment, the *ABCA4* retinopathy is Stargardt disease.

[0023] In one aspect, there is provided a method of treating a *ABCA4* retinopathy comprising administering to a subject a composition comprising a modified U1 snRNA as described herein and a nucleic acid construct as described herein.

[0024] In one embodiment, the *ABCA4* retinopathy is Stargardt disease.

[0025] In one aspect, there is provided a pharmaceutical composition comprising (a) a therapeutically effective amount of a modified U1 snRNA as described herein or a nucleic acid construct as described herein and (b) one or more pharmaceutically acceptable carriers and/or diluents.

[0026] In another aspect, there is provided a method of retaining exon 40 in a mature mRNA transcript of a mutated *ABCA4* gene, wherein the mutated *ABCA4* gene comprises a mutation that induces skipping of exon 40, wherein the mutation is located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40, the method comprising providing a modified U1 snRNA as described herein, wherein binding of the modified U1 snRNA to the target sequence induces the retention of exon 40 in a mature mRNA transcript of the mutated *ABCA4* gene.

[0027] In one embodiment, the mutation that induces skipping of exon 40 is c.5714+5G>A.

[0028] In one embodiment, the method as described herein comprises providing a modified U1 snRNA having a single-stranded binding nucleotide sequence that is between 11 and 21 nucleotides in length.

[0029] In one embodiment, the method as described herein comprises providing a modified U1 snRNA having a single-stranded binding nucleotide sequence selected from the group consisting of SEQ ID NOs 2 to 19.

[0030] In one embodiment, the method as described herein comprises providing a modified U1 snRNA having a single-stranded binding nucleotide sequence selected from the group consisting of SEQ ID NOs 7, 8 and 10.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0032] **Fig. 1** shows a representation of an engineered U1-snRNA whose antisense sequence has been adapted to tolerate a 5'-SS mutation and to rescue correct exon splicing. Aberrant splicing is indicated in black, rescued splicing is indicated in grey. The structure of the DNA construct expressing the engineered U1-snRNA is indicated at the top-right.

[0033] **Fig. 2** shows A) Design of ABCA4 wild-type and mutant minigenes. Exons and introns are indicated as thick and thin boxes respectively. The splicing pattern is indicated through black arcs. B) Analysis of ABCA4 splicing in the minigene systems by RT-FLA-PCR. The wild-type (wt) and mutant (mt) minigenes are transfected into 293T cells, along with the control condition (no transfection). The primers used for the PCR are indicated in Figure 2A (black arrows). The PCR products are analyzed though a 2.3% agarose gel. The band size ladder is indicated on the left, the transcripts corresponding to each band are indicated on the right.

[0034] **Fig. 3** shows A) Design of tiling U1s targeting a region around exon 40 5'-SS, with the 5'-most position of the target sequence spanning from position -13 to +5. B) Rescuing of exon 40 splicing by tiling U1s. The mutant minigene carrying the mutation c.5714+5G>A ("mut") was transfected in 293T cells along with control constructs ("O" or "U1", corresponding to the empty vector and the endogenous U1 respectively), U1* (wild-type U1 whose sequence is complementary to "A" at c.5714+5), or with each one of the U1s targeting different positions (the number indicates the 5'-most position of the target sequence). Untransfected cells ("no") and cells transfected with the wild-type minigene ("wt") were used as a control. Exon 40 inclusion and skipping, as well as the overall transcript expression, were measured through splice site-specific qRT-PCR. The primers used for the PCR (1+2 for exon inclusion, 1+3 for exon skipping and 4+5 for the total transcript) are indicated in Figure 3A as black arrows. Values are normalized through GAPDH expression and indicated as fold-change referred to the sample with the mutant minigene only. Mean and standard deviation were calculated from 4 different replicates.

[0035] **Fig. 4** shows A) Selection of the 3 best U1s (dark grey), namely "-8", "-7" and "-5". B) Titration of the 3 best U1s. 293T cells were transfected with the mutant minigene and increasing concentrations of the respective U1. The control U1 was co-transfected along with the therapeutic U1 in order to maintain the same amount of transfected plasmid in all conditions. Exon 40 inclusion and skipping were measured through splice site-specific qRT-PCR. Values are normalized to the sample treated with 0 nM U1. C) The same U1s were tested with the wild-type minigene. Exon 40 inclusion and overall transcript expression were measured through splice site-specific qRT-PCR. Transfection and qRT-PCR were performed both in (B) and (C) as described for Figure 3B. The primers used for the PCR (1+2 for exon inclusion, 1+3 for exon skipping and 4+5 for the total transcript) are indicated in Figure 4A as black arrows. Values are normalized through GAPDH expression and indicated as fold-change compared to the control sample. Mean and standard deviation were calculated from 4 different replicates.

[0036] **Fig. 5** shows delivery of lentiviral U1 for rescuing correct ABCA4 splicing in CRISPR-edited RPE cells carrying the mutation c.5714+5G>A. The results are obtained from two replicate experiments in which mutant RPE cells (C27) were infected with lentiviral particles expressing either the control U1 or "-7". Non-infected wild-type (H9) and C27 cells were also analyzed as a control. The cells were further treated with CHX (or DMSO as a control) in order to prevent nonsense-mediated decay (NMD) of transcripts without exon 40 (due to the premature termination codons generated from the frameshift). The RT-FLA-PCR was performed as described in Figure 2B. The transcripts corresponding to each band are indicated on the left.

[0037] **Fig. 6** shows predicted U1 secondary structures for rescuing correct splicing of ABCA4 carrying the mutation c.5714+5G>A. Structures were predicted by RNAfold in ViennaRNA package using default settings. A) The secondary structures for the endogenous U1 (top), the top 3 U1 leads ("-8", "-7", and "-5"; middle row) and representative U1s with relatively lower efficiency ("-13", "-10" and "-2"; bottom row) were depicted. B) The secondary structures of representative U1s with relatively lower efficiency ("-9", "+4" and "+5") that manifest similar structures as the endogenous U1 were depicted.

[0038] **Fig. 7** shows the average co-transcriptional binding inaccessibility of U1 target sites. The co-transcriptional structures of exon 40 and 50 bases at each of its flanking intronic sequence in both c.5714+5G>A and wild-type (WT) pre-mRNA were predicted. Based on the structures, the average co-transcriptional binding inaccessibility of each U1 target site considered here that spans from -13 to +15 was determined by averaging the co-transcriptional binding inaccessibility of the 11 bases in a target site. For example, a higher inaccessibility score at -3 obtained in c.5714+5G>A suggests that the U1 target site whose 5'-most position of the target sequence is at -3 is more accessible for Watson-Crick base pairing to U1 compared to the wild-type pre-mRNA.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0039] In one aspect, the present invention provides a modified U1 small nuclear RNA (snRNA) configured to retain exon 40 in a mature mRNA transcript of a mutated ABCA4 gene, the mutated ABCA4 gene comprising a mutation that induces skipping of exon 40, wherein the mutation is located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40, wherein the modification comprises replacing a portion of a single-stranded nucleotide sequence of a 5' region of a wild-type U1 snRNA with a single-stranded binding nucleotide sequence capable of hybridizing to a target sequence present on a pre-mRNA transcript of the mutated ABCA4 gene, and wherein the

target sequence is located in a region between 13 base pairs upstream and 15 base pairs downstream of the donor splice site of exon 40.

[0040] While the modified U1 snRNA can retain exon 40 in a mature mRNA transcript of a mutated ABCA4 gene having a mutation located anywhere between positions -3 and +8, the modified U1 snRNA need not necessarily bind between positions -3 and +8 to carry out this function of retaining exon 40. This function can also be effected by the modified U1 snRNA binding to sites adjacent to the mutation and within the target sequence which is located between positions -13 and +15.

[0041] As used herein, the terms “wild-type U1 snRNA” and “endogenous U1 snRNA” are used interchangeably.

[0042] During post-transcriptional processing of wild-type ABCA4 gene, exon 40 is present in the mature mRNA transcript of *ABCA4*. Mutations at or near the donor splice site in a mutated *ABCA4* gene may result in the skipping of exon 40 due to aberrant splicing. The modified U1 snRNA of the present embodiments is configured to restore wild-type splicing of the mutated *ABCA4* transcript, thereby retaining exon 40 (i.e. rescue exon skipping of exon 40) in a mature mRNA transcript of a mutated *ABCA4* gene.

[0043] As used herein, the terms “donor splice site” and “5’ splice site” are used interchangeably.

[0044] As used herein, the terms “U1” and “U1 snRNA” are used interchangeably.

[0045] As used herein, the term “modified U1 snRNA” is meant to refer to a U1 snRNA that is not a wild-type U1 snRNA. The terms “modified U1 snRNA”, “engineered U1 snRNA” and “adapted U1 snRNA” are used interchangeably. The modified U1 snRNA may be characterized by the position relative to the donor splice site that it targets. For example, a “-8” U1 snRNA refers to a U1 snRNA whose target sequence has its 5’-most end at position “-8” (i.e. 8 base pairs upstream) from the donor splice site of the target exon. The “-8” position of the donor splice site of the target exon refers to the 8^{th}-last base of the exon.

[0046] In one embodiment, the coordinates of exon 40 are chr1:94,010,800-94,010,929 in GRCh38/hg38. In one embodiment, the position of the donor splice site (1 nucleotide upstream of the splice junction) of exon 40 based on NM_000350.3(ABCA4) is: 5817.

[0047] In one embodiment, the mutation that induces skipping of exon 40 is c.5714+5G>A. The coordinates c.5714+5G>A were documented in Cremers at al. (1998), Human Molecular Genetics, 1998, Vol. 7, No. 3. The actual genomic coordinates of c.5714+5G>A based on the current reference sequence NM_000350.3(ABCA4) are chr1:94,010,795-94,010,795 (GRCh38). However, the term “c.5714+5G>A” has been used in this specification since this nomenclature is commonly used in bibliography and public

repositories such as ClinVar. As such, a person skilled in the art would be able to understand the position of the mutation that “c.5714+5G>A” refers to.

[0048] It is commonly known in the art that U1 snRNAs are highly tolerant of mismatches with their target sequence without loss of their biological function. Therefore, it would be generally appreciated by a person skilled in the art that the modified U1 snRNAs of the present embodiments would work for any mutation located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40.

[0049] In one embodiment, the region comprises the sequence of SEQ ID NO: 1. The target sequence may be located anywhere within this region, which corresponds to between 13 base pairs upstream and 15 base pairs downstream of a donor splice site of exon 40.

[0050] It should be noted that not all regions capable of being bound to modified U1 snRNAs can produce the desired effect of rescuing splicing by preventing exon skipping. In particular, as current knowledge is limited in predicting the range of U1 target region (i.e. “distance” of the U1 target region from the 5’ splice site and the mutation loci) for which a modified U1 is effective, screening is required to identify the target region. While the binding position of the modified U1 snRNAs and the loci and type of mutation may determine their efficacy (also known as effectiveness or the ability to prevent exon skipping), the efficiency (which is determined by comparing exon inclusion and exon skipping) of the U1 snRNAs would depend on factors such as the secondary structure of the modified U1 snRNAs, the extent of conservation of donor splice site sequence, local pre-mRNA secondary structure at the target exon (to facilitate binding, recruitment and assembly of components of the spliceosome) and presence of sequence motifs used by RNA-binding proteins for splicing regulation. Advantageously, the modified U1 snRNAs of the present embodiments that bind to the identified target region from -13 to +15 are effective in preventing exon skipping of exon 40 and have been shown to prevent exon skipping of exon 40 with greater efficiency than the wild-type U1 snRNA.

[0051] In one embodiment, the single-stranded binding nucleotide sequence is between 11 and 21 nucleotides in length. Preferably, the single-stranded binding nucleotide sequence is 11 nucleotides in length. In the context of U1 snRNA, the terms “single-stranded binding nucleotide sequence” and “custom sequence” are used interchangeably.

[0052] In one embodiment, the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 2 to 19. In one embodiment, the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 7, 8 and 10.

[0053] In another aspect, the present invention provides a nucleic acid construct comprising a polynucleotide sequence encoding the modified U1 snRNA as described herein.

[0054] In one embodiment, the polynucleotide sequence encoding the modified U1 snRNA is operably linked to a RNU1-1 promoter and to a RNU1-1 terminator.

[0055] In one embodiment, the nucleic acid construct as described herein comprises the sequence of SEQ ID NO: 25.

[0056] In one embodiment, a portion of the polynucleotide sequence that encodes the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 26 to 43. In one embodiment, the portion of the polynucleotide sequence that encodes the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 31, 32 and 34.

[0057] In one embodiment, the nucleic acid construct as described herein is contained in an expression vector.

[0058] As used herein, the term “expression vector” is meant to include a vector, plasmid or vehicle designed to enable the expression of an inserted nucleic acid sequence following transformation into the host.

[0059] In various embodiments, the expression vector is selected from the group consisting of a lentiviral vector and an adeno-associated viral vector.

[0060] In another embodiment, the nucleic acid construct as described herein is contained in a lipid nanoparticle.

[0061] Lipid nanoparticles as used herein may refer to carrier systems in the nanometer size comprising a continuous aqueous phase and at least one dispersed oily phase, in which the oily phase comprises at least one amphiphilic lipid such as phospholipids and at least one solubilizing lipid with a monolayer around an amorphous core. Lipid nanoparticles are known for their high degree of biocompatibility, controlled release, efficient targeting, stability, natural biodegradability and high therapeutic index to their payload. Lipid nanoparticles may be assembled as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and NanoSpheres (NS). The lipids used in the process of synthesizing the lipid nanoparticle compositions may include fatty acids, triglycerides, triacylglycerols, acylglycerols, fats, waxes, cholesterol, sphingolipids, glycerides, sterides, cerides, glycolipids, sulfolipids, lipoproteins, chylomicrons and the derivatives of these lipids. Surfactants used in the assembly of lipid nanoparticles may include biocompatible and biodegradable surfactants such lecithins, polysorbates, monoglycerides, diglycerides,

triglycerides, glyceryl oleate, polaxamers and other non-toxic, non-ionic surfactants that are known in the art.

[0062] In one aspect, there is provided a modified U1 snRNA as described herein or a nucleic acid construct as described herein for use in treating a ABCA4 retinopathy.

[0063] As used herein, “ABCA4 retinopathy” refers to retinal diseases caused by pathogenic ABCA4 mutations. Loss-of-function mutations in ABCA4 can cause Stargardt disease, con-rod dystrophy, retinitis pigmentosa, and bull eye maculopathy.

[0064] As used herein, the terms “treat”, “treating” or “treatment” in the context of a disease includes any regimen for the prophylaxis or treatment of the disease. This includes improving the clinical outcomes and/or survival rates of patients having the disease.

[0065] These modified U1 snRNAs or nucleic acid constructs may be used in compositions that can be used for treatment, e.g. as a pharmaceutical composition comprising the modified U1 snRNA or nucleic acid construct of the present embodiments and pharmaceutically acceptable carrier. The carrier is selected from the group consisting of a nanoparticle, such as a polymeric nanoparticle; a liposome, such as pH-sensitive liposome, an antibody conjugated liposome; a viral vector, a cationic lipid, a polymer, and a cell penetrating peptide.

[0066] In one embodiment, the *ABCA4* retinopathy is Stargardt disease.

[0067] In another aspect, there is provided a use of a modified U1 snRNA as described herein and a nucleic acid construct as described herein in the manufacture of a medicament for treating a *ABCA4* retinopathy.

[0068] In one embodiment, the *ABCA4* retinopathy is Stargardt disease.

[0069] In one aspect, there is provided a method of treating a *ABCA4* retinopathy comprising administering to a subject a composition comprising a modified U1 snRNA as described herein and a nucleic acid construct as described herein.

[0070] In one embodiment, the *ABCA4* retinopathy is Stargardt disease.

[0071] In one aspect, there is provided a pharmaceutical composition comprising (a) a therapeutically effective amount of a modified U1 snRNA as described herein or a nucleic acid construct as described herein and (b) one or more pharmaceutically acceptable carriers and/or diluents.

[0072] The term “therapeutically effective amount” refers to the amount of the modified U1 snRNA as described herein that is required to confer the intended therapeutic effect in the subject, which amount will vary depending on the route of administration, status of disease, and possible inclusion of other therapeutics or excipients. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time

necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the therapeutic agent to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the protein or protein portion are outweighed by the therapeutically beneficial effects.

[0073] A "therapeutically effective amount" for therapy may also be measured by its ability to stabilize the progression of disease. A therapeutically effective amount of a therapeutic agent may reduce or ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[0074] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition, and/or prevent the worsening of symptoms associated with the disease or condition. An example of a positive therapeutic response in the context of retinal degenerative diseases is the halting or delaying of progression to blindness. Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[0075] As used herein, the term "pharmaceutical composition" is meant to include any pharmaceutical preparation or formulation which is suitable for administration to a subject in need thereof. The composition may be suitable for parenteral administration either naked or complexed with a delivery agent to a patient. The carrier is selected from the group consisting of a nanoparticle, such as a polymeric nanoparticle; a liposome, such as pH-sensitive liposome, an antibody conjugated liposome; a viral vector, a cationic lipid, a polymer, and a cell penetrating peptide. It will be appreciated that pharmaceutical compositions provided according to the disclosure may be administered by any means known in the art. The pharmaceutical composition may be administered orally, or rectal, or transmucosal, or intestinal, or intramuscular, or subcutaneous, or intramedullary, or intrathecal, or direct intraventricular, or intravenous, or intravitreal, or intraperitoneal, or intranasal, or intraocular. Preferably, the pharmaceutical composition is administered

intraocularly or intravitreally. Multiple injections of the pharmaceutical composition may be administered at one time to boost the dosage of modified U1 snRNAs administered.

[0076] A pharmaceutically acceptable carrier refers, generally, to materials that are suitable for administration to a subject wherein the carrier is not biologically harmful, or otherwise, causes undesirable effects. Such carriers are typically inert ingredients of a medicament. Typically a carrier is administered to a subject along with an active ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of a pharmaceutical composition in which it is contained.

[0077] The pharmaceutical compositions or formulations of the disclosure, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0078] Combination therapy with an additional therapeutic agent may also contemplated by the disclosure. The term "combination" or "combination therapy" as used throughout the specification, is meant to encompass the administration of the referred therapeutic agents to a subject suffering from a disease, disorder or pathological condition, in the same or separate pharmaceutical formulations, and at the same time or at different times. If the therapeutic agents are administered at different times they should be administered sufficiently close in time to provide for the potentiating or synergistic response to occur. In such instances, it is contemplated that one would typically administer both therapeutic agents within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1 , 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations. In other situations, it might be desirable to reduce the time between administration, administering both therapeutic agents within seconds or minutes to hours, preferably within about 6 hours from each other, more preferably within about 1 or 3 hours.

[0079] To practice the methods of this invention, the modified U1 snRNA as described herein may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally or via an implanted reservoir. The term “parenteral” as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, and intracranial injection or infusion

techniques. A sterile injectable composition, e.g., a sterile injectable aqueous or oleaginous suspension, can be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as Tween 80) and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluents or solvent for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are mannitol, water, Ringer's Solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium (eg. Synthetic mono-or dyglycerides). Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long-chain alcohol diluents or dispersant, or carboxymethyl cellulose or similar dispersing agents. Other commonly used surfactants such as Tweens or Spans or other similar emulsifying agents or bioavailablity enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms can also be used for the purposes of formulation.

[0080] A composition for oral administration can be any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions or emulsions are administered orally, the active ingredient can be suspended or dissolved in an oily phase combined with emulsifying or suspending agents. If desired, certain sweetening, flavouring, or colouring agents can be added. A nasal aerosol or inhalation composition can be prepared according to techniques well known in the art of pharmaceutical formulation. A modified U1 snRNA-containing composition can also be administered in the form of suppositories for rectal administration. The carrier in the pharmaceutical composition must be "acceptable" in the sense of being compatible with the active ingredient of the formulation (and preferable, capable of stabilising it) and not deleterious to the subject to be treated. For example, one or more solubilising agents, which form more soluble complexes with the modified U1 snRNAs, or more solubilising agents, can be utilised as pharmaceutical carriers for delivery of the active compounds. Examples of other carriers include colloidal silicon dioxide, magnesium stearate, sodium lauryl sulphate, and D&C Yellow #10.

[0081] In another aspect, there is provided a method of retaining exon 40 in a mature mRNA transcript of a mutated *ABCA4* gene, wherein the mutated *ABCA4* gene comprises a mutation that induces skipping of exon 40, wherein the mutation is located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40, the method comprising providing a modified U1 snRNA as described herein, wherein binding of the modified U1 snRNA to the target sequence induces the retention of exon 40 in a mature mRNA transcript of the mutated *ABCA4* gene.

[0082] In one embodiment, the mutation that induces skipping of exon 40 is c.5714+5G>A.

[0083] In one embodiment, the method as described herein comprises providing a modified U1 snRNA having a single-stranded binding nucleotide sequence that is between 11 and 21 nucleotides in length. Preferably, the single-stranded binding nucleotide sequence is 11 nucleotides in length.

[0084] In one embodiment, the method as described herein comprises providing a modified U1 snRNA having a single-stranded binding nucleotide sequence selected from the group consisting of SEQ ID NOs 2 to 19. In one embodiment, the method as described herein comprises providing a modified U1 snRNA having a single-stranded binding nucleotide sequence selected from the group consisting of SEQ ID NOs 7, 8 and 10.

[0085] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0086] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0087] Other embodiments are within the following claims and non- limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0088] **MATERIALS AND METHODS**

[0089] **Plasmid construct**

[0090] The lentiviral plasmid delivering the engineered U1s was obtained by substituting the sgRNA cassette of the pLKO5.sgRNA.EFS.GFP plasmid (Addgene #57822) with the human U1 gene sequence (including both promoter, U1 sequence and terminator). The cloning site for engineered U1s was later introduced by replacing the antisense region of the endogenous U1 sequence. The AAV equivalent of the plasmid was obtained by inserting the U1 cassette of the lentiviral vector (including both promoter, cloning site, U1 scaffold sequence and terminator) in the NheI cloning site of the pAAV.CMV.PI.EGFP.WPRE.bGH plasmid (Addgene #105530). Cloning reagents were provided by New England Biolabs.

[0091] **Cell cultures**

[0092] HEK293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Cytiva #SH30022.01) supplemented with 10% fetal bovine serum (FBS, Cytiva #SV30160.03) and 1% penicillin/streptomycin (Gibco #15140-122) in a humidified incubator with 5% CO2 at 37°C. The cells were passaged every 2-3 days by trypsinization with 0.05% trypsin-EDTA solution (Gibco #25200-072). Retina pigment epithelium (RPE) cells were obtained by differentiating wild-type or CRISPR-edited human embryonic stem cells (H9), following the protocol published by Farnoodian et al. (2022, doi.org/10.1016/j.stemcr.2022.10.001). RPE cells were cultured with mTeSR1 medium in Matrigel-coated dishes, using a humidified incubator with 5% CO2 at 37°C. The medium was changed every 2-3 days. All experiments were performed with cells between passages 2 and 8.

[0093] **Cell transfection**

[0094] HEK293T cells were seeded in 24-well plates at a density of 140,000 cells per well and cultured overnight. For each well, 500 ng of plasmid DNA (280 ng of minigene + 220 ng of lentiviral U1 plasmid), 1.5 µL of Lipofectamine 3000 reagent, and 1.0 µL of P3000 reagent (Invitrogen #L3000-015) were diluted in 50 µL of Opti-MEM medium (Gibco # 31985-070), mixed and incubated for 20 minutes at room temperature. The total amount of lentiviral U1 plasmid was increased to 440 ng in the case of the titration experiments. The mixture was added to the cells (cultured in 450 µL of DMEM without penicillin/streptomycin)

and incubated for 6 hours before replacing the medium with fresh DMEM. The cells were harvested after 24 hours of incubation via TRIzol™ Reagent (Invitrogen #15596026).

[0095] **Cell infection**

[0096] Lentiviral particles were produced in HEK293T cells (4X10^6 cells per 10 cm plate) by transfection of the lentiviral U1 plasmid together with the packaging plasmids. Transfection was performed as described above, using for each plate 5274.6 ng of U1 plasmid, 6124.2 ng of pMDLg-pRRE (Addgene #12251), 2879.2 ng of pRSV-Rev (Addgene #12253), 4012 ng of pMD2.G (Addgene #12259), 45 µL of Lipofectamine 3000 reagent, and 30 µL of P3000 reagent diluted in 1.5 mL of Opti-MEM medium. The mixture was added to the cells (cultured in 13.5 mL of DMEM without antibiotics) and incubated for 6 hours before replacing the medium with 6 mL of fresh DMEM. The medium containing the lentiviral particles was collected 24, 48 and 72 hours after transfection and stored at 4ºC, replacing it with 6 mL of fresh DMEM at each time. RPE cells were infected 96 hours after they had been seeded (550,000 cells per well of a 12-well plate), exactly after the 3rd collection of lentiviral particles (72 hours from HEK293T transfection). Lentiviral particles were filtered with 40 µm filter, centrifuged at 23,000 rpm for 2 hours at 16ºC and resuspended with 787.5 of HBSS 1X (HyClone #SH30588.01). The lentiviral solution was applied to RPE cells (cultured in 4015 uL of medium + 1 µg/mL of polybrene) in a way to reach ~70-90 % of infection rate (checked by fluorescence-activated cell sorting). The infected cells were harvested after 72 hours of incubation via TRIzol™ Reagent.

[0097] **RNA and cDNA samples**

[0098] Total RNA was extracted from cultured cells using TRIzol™ Reagent following the manufacturer's instructions. Briefly, the cells were harvested and lysed with TRIzol™, and chloroform was added to separate the RNA-containing aqueous phase. The RNA was precipitated with isopropanol and washed with 75% ethanol. The RNA pellet was dissolved in nuclease-free water and the concentration was measured by spectrophotometry. cDNA was synthesized from 1 µg of RNA using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific #EP0751) following the manufacturer's protocol.

[0099] **FLA-PCR and qRT-PCR**

[00100] cDNA was used as a template for fragment-length-analysis (FLA) PCR with DreamTaq DNA polymerase. The PCR reaction mixture consisted of 1X DreamTaq™ Green buffer (Thermo Fisher Scientific #K1081), 0.2 µM of forward and reverse primers, and 60 ng of cDNA in a final volume of 50 µL. The PCR program consisted of initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. The final extension was

performed at 72°C for 5 minutes. PCR products were analyzed by gel electrophoresis on 2.3% agarose gel. qPCR was performed using 10 ng of cDNA, 0.4 µM of forward and reverse primers, and PowerUp™ SYBR Green PCR (Thermo Fisher Scientific #A25741). The qPCR program consisted of pre-heating at 50°C for 2 minutes, initial denaturation at 95°C for 90 seconds, followed by 39 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 40 seconds. The primers' melting curve was measured by increasing the temperature from 65 to 95°C by 0.5°C every 5 seconds. The qPCR reaction was performed with a CFX96™ Real-Time System (BIO-RAD). The following primers were used: FLA-PCR primers, CGCTGCTCAGGTTCAACGCCGTGC (SEQ ID NO: 44) + GACACACAGCCTGTCCACT (SEQ ID NO: 45); qPCR primer 1, CGCTGCTCAGGTTCAACGCCGTGC (SEQ ID NO: 46); qPCR primer 2, GCAGAGTGCTCCTCACCAAAC (SEQ ID NO: 47); qPCR primer 3, TGGGCTCGGCAATCCAAAC (SEQ ID NO: 48); qPCR primer 4, AGGAGCCCATTGTTGATGAA (SEQ ID NO: 49); qPCR primer 5, GGTGCCTGGATAAATCTTGGT (SEQ ID NO: 50); qPCR primers for GAPDH, GCAAATTCCATGGCACCGT (SEQ ID NO: 51) + GCCCCACTTGATTTTGGAGG (SEQ ID NO: 52).

[00101] **Method for predicting co-transcriptional structures and average co-transcriptional binding inaccessibility**

[00102] A ‘‘window of analysis’’ of pre-determined sequence length of 1500 nucleotides that includes the full length of exon 40 and 50 bases of its flanking intronic sequence corresponds to a ‘‘step of transcriptional analysis’’. To approximate the transcription elongation process, the window of analysis is shifted one nucleotide at a time along the pre-mRNA sequence towards the 3’ end. At each step of transcriptional analysis, the possible secondary structures for the window sequence are predicted with mfold. Since it was highly probable that the nascent pre-mRNA may not have the chance to assume optimal structures, sub-optimal structures whose energies lie within 5% of the optimum were accepted. Subsequently, each nucleotide within the U1 target site was scored for binding inaccessibility based on whether it is paired in the predicted secondary structures.

[00103] **EXAMPLES**

[00104] Non-limiting examples of the invention and comparative examples will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

[00105] Example 1

[00106] Genetic mutations resulting in the loss of donor splice sites, also known at 5' splice sites (5'-SSs) can lead to aberrant splicing of the nascent mRNA, including exclusion of exon, activation of alternate or cryptic splice sites, or intron retention. Depending on the specific mis-splicing event, the resultant mature mRNA typically undergoes significant alteration in its coding sequence and/or shifting of its codon reading frame, any of which causes loss of normal function and expression of protein. The 5'-SSs are crucial for correct exon splicing mediated by the U1-snRNA (U1 small nuclear RNA), a small nuclear RNA whose main function is to anneal to 5'-SSs and initiate splicing. Therapeutic U1s can be designed to negate the loss of donor splice sites and rescue exon splicing. In the present study, engineered U1s were developed as a potential therapeutic strategy for Stargardt disease, an inherited retinopathy that leads to progressive loss of vision with no therapy available. This mutation induces exon skipping in a key regulator of the visual cycle, whose consequent loss of function is responsible for the accumulation of toxic compounds and cell death in the retina. A library of U1-snRNAs was designed and validated through a minigene system to efficiently restore wild type splicing of the mutated transcript. U1 efficiency was improved by selecting optimal target positions in proximity of the 5'-SS rather than the canonical U1 binding site, a mechanism known as "splice-site selection from a distance". In addition, it was found that U1s designed to target different sequences can display a variable range of predicted off-targets, with the possibility to improve precision along with efficiency.

[00107] The technology disclosed here consists of specific compositions of DNA molecules that serve as templates to express engineered U1-snRNAs (U1 small nuclear RNAs). The engineered U1 is designed to restore loss of exon due to mis-splicing of the *ABCA4* nascent mRNA (a.k.a. pre-mRNA) resulting from the loss-of-function c.5714+5G>A genetic mutation. By correcting splicing, the approach restores the expression of wild-type ABCA4 protein that can be a potential therapeutic strategy for the treatment of ABCA4 retinopathies.

[00108] Example 2

[00109] U1-snRNAs (U1 small nuclear RNA) are endogenously expressed in every cell to mediate exon splicing by binding to donor splice sites (5'-SSs) and recruits the splicing machinery. This essential process is lost when a genetic mutation at a donor splice site abrogates endogenous U1s binding, and typically leads to aberrant splicing of the nascent mRNA including exclusion of exon, activation of alternate or cryptic splice sites, or intron retention. Depending on the specific mis-splicing event, the resultant mature mRNA typically undergoes significant alteration in its coding sequence and/or shifting of its codon reading frame, any of which causes loss of normal function and expression of protein. As the 5'-SSs

are crucial for correct exon splicing mediated by the U1-snRNA, therapeutic U1s can be designed to negate the loss of donor splice sites and rescue exon splicing.

[00110] The pathogenic NM_000350.3(ABCA4):c.5714+5G>A genotype is the most common genetic mutation in the Greek population, which has been demonstrated to induce the skipping of exon 40 (chr1:94,010,800-94,010,929 in GRCh38/hg38). Transcripts without exon 40 are frameshifted and predisposed to degradation via the nonsense-mediated decay pathway, which results in the loss of functional ABCA4 protein that is essential for maintaining a healthy retina. A minigene system was designed in order to assess the effect of c.5714+5G>A on ABCA4 splicing (Figure 2A). The minigene consists of a DNA plasmid expressing a transcript containing ABCA4 exons 39, 40, 41 and 42, along with part of intron 38 (the last 522 bp) and the full sequence of introns 39, 40 and 41 (comprehensive coordinates: chr1:94,008,235- 94,011,907). Two versions of the minigene were developed, one carrying the wild-type sequence and one carrying the mutation c.5714+5G>A, which is located in intron 40 and lies 5 bases from exon 40 5'-SS (denoted with a red asterisk in Figure 2A). The minigenes were tested in 293T cells through reverse-transcription fragment-length-analysis PCR (RT-FLA-PCR) followed by Sanger sequencing. Consistently with previous results, the wild-type minigene expressed a full transcript (which includes exons 39, 40, 41 and 42) while the mutant minigene expressed the full transcript and a major transcript without exon 40 (Figure 2B).

[00111] Example 3

[00112] The technology disclosed here consists of specific compositions of DNA that express engineered U1-snRNAs in target cells and tissue. The engineered U1-snRNAs retains the endogenous function to mediate exon splicing and are designed to bind within exon 40, across exon 40 and intron 40, or within intron 40. By doing so, the splicing machinery will be recruited and thereby leading to exon inclusion that is otherwise skipped (Figure 1). The DNA sequence producing and delivering the U1-snRNAs is composed of the following elements (from 5' to 3' direction of the coding strand, Figure 1):

1) The promoter of the human gene RNU1-1, having coordinates chr1:16,514,286-16,514,678 (GRCh38/hg38, reverse strand).

2) The cassette coding for the U1-snRNA. The cassette is then composed of the customized sequence that anneals to the target nascent mRNA molecule (ABCA4), followed by the scaffold sequence of the human gene RNU1-1, having coordinates chr1:16,514,122-16,514,274 (GRCh38/hg38, reverse strand). The sequence of the customized region varies according with the position of the target site.

3) The terminator of the human gene RNU1-1, having coordinates chr1:16,514,085-16,514,121 (GRCh38/hg38, reverse strand).

[00113] Example 4

[00114] Multiple U1-snRNAs each targeting a different position along the mutated 5'-splice site (5'-SS) were designed and tested in the above mentioned minigene model (Figure 3). The mutant minigene was transfected into 293T cells along with the plasmid expressing a specific U1. The inclusion of exon 40 was quantitated through splice site-specific qRT-PCR. Results showed that the entire exon 40 is correctly and efficiently spliced in by 3 different U1s (Figure 4B), which respectively target the following positions (positions are referred to the 5'-SS of the skipped exon): from -8 to +3, from -7 to +4 and from +1 to +11.

[00115] Example 5

[00116] The best 3 U1-RNAs candidates, namely "-8", "-7" and "-5" (Figure 4A), were subsequently selected for dose response studies in rescuing exon 40 splicing (Figure 4B). They were each further tested on the wild-type minigene (that is, no loss of 5'-SS) as a control for possible off-target effects, and no effect on exon 40 splicing was observed, indicating each of the U1 candidates is selective only to the mutation (Figure 4C).

[00117] Example 6

[00118] To demonstrate the rescue of exon 40 by the present U1 candidates in retinal cells, an iPSC-RPE cell line was developed. Briefly, wildtype human embryonic stem cells were CRISPR-Cas9 edited to introduce the c.5714+5G>A mutation. A single clonal line carrying the homozygous mutation, indicated as C27, was isolated and thereafter differentiated to RPE cells, which represent a validated in vitro model for Stargardt disease. As expected, exon 40 was skipped in the mutant line and not in the wildtype cells. Given that the top 3 U1 candidates showed similar dose responses, one representative candidate namely "-7", was selected for further validation. The U1 was delivered by a lentiviral system that was encoded within the plasmid expressing either the control or "-7" U1. iPSC-RPE cells were infected with the lentiviral particles, and the resultant effect of ABCA4 expression and exon 40 splicing were assessed through RT-FLA-PCR. Only "-7" and not the control U1 was found to restore exon 40 skipping, which corroborates with the effect observed in 293T cells (Figure 5).

[00119] Of note, the DNA sequence coding for the engineered U1-snRNA can be potentially integrated into different delivery systems, including lentivirus and adeno-associated virus, therefore providing a discrete level of flexibility. In addition, although these U1-snRNAs were tested for mutation c.5714+5G>A, they could potentially be used for any other mutation affecting the same donor splice site of the same exon.

[00120] Example 7

[00121] The manifestation of a modified U1 to the endogenous U1 secondary structures is a necessary but not sufficient factor in the determination of its efficiency in restoring exon 40 inclusion. Modified U1 snRNAs may not fold into secondary structures that are similar to the endogenous U1 snRNA. Figure 6A shows that the top 3 U1 leads fold into secondary structures that are similar to the endogenous U1, whereas U1s with relatively lower efficiency do not. Based on Figure 6A, modified U1 snRNAs with higher efficiency may tend to have a secondary structure similar to the endogenous U1 snRNA. Figure 6B shows that even when a modified U1 folds into secondary structures similar to the endogenous U1, high efficiency is not guaranteed, which suggests that other factors are also important. In conclusion, the identification of a highly efficacious and efficient U1 for therapeutic application is not intuitive to a skilled person as current knowledge limits the prediction of a modified U1's efficiency.

[00122] Example 8

[00123] As shown in Figure 7, a single G-to-A transition in c.5714+5G>A resulted in a lower co-transcriptional inaccessibility than the WT at U1 target sites from -10 onwards. This higher co-transcriptional accessibility may have contributed to the efficacy and efficiency of the modified U1s of the present invention in rescuing exon 40 inclusion. The presence of RNA-BP motifs within -13 to +15 in both c.5714+5G>A and WT pre-mRNA was determined. Notably, a SRSF2 motif from +4 to +11 was abrogated by the G-to-A mutation that is otherwise present in the WT pre-mRNA. As SRSF2 is a potent regulator of exon inclusion, the loss of its binding motif, due to the c.5714+5G>A mutation, within the U1 target sites considered here suggests that the U1 can reconstitute the splicing enhancer role of SRSF2.

[00124] Example 9

[00125] Table 1 shows the sequences of the present embodiments.

[00126] SEQ ID NO: 20 is a combination of SEQ ID NOs 21 to 24. SEQ ID NO: 25 is identical to SEQ ID NO: 20, except that the cloning site (SEQ ID NO: 22) is replaced by "NNNNNNNNNNN" in SEQ ID NO: 25. The sequence "NNNNNNNNNNN" in SEQ ID NO: 25 represents any one of SEQ ID NOs 26 to 43.

SEQ ID NO	Description of sequence	5' to 3'
1	Region*	TCCTCTCCCAATGgtacatccatgccac
2	U1 snRNA single-stranded binding nucleotide sequence annealing from position -13 to -3	UUGGGAGAGGA
3	U1 snRNA single-stranded binding nucleotide sequence annealing from position -12 to -2	AUUGGGAGAGG
4	U1 snRNA single-stranded binding	CAUUGGGAGAG

	nucleotide sequence annealing from position -11 to -1	
5	U1 snRNA single-stranded binding nucleotide sequence annealing from position -10 to +1	CCAUUGGGAGA
6	U1 snRNA single-stranded binding nucleotide sequence annealing from position -9 to +2	ACCAUUGGGAG
7	U1 snRNA single-stranded binding nucleotide sequence annealing from position -8 to +3	UACCAUUGGGA
8	U1 snRNA single-stranded binding nucleotide sequence annealing from position -7 to +4	GUACCAUUGGG
9	U1 snRNA single-stranded binding nucleotide sequence annealing from position -6 to +5**	uGUACCAUUGG
10	U1 snRNA single-stranded binding nucleotide sequence annealing from position -5 to +6**	AuGUACCAUUG
11	U1 snRNA single-stranded binding nucleotide sequence annealing from position -4 to +7**	GAuGUACCAUU
12	U1 snRNA single-stranded binding nucleotide sequence annealing from position -3 to +8**	GGAuGUACCAU
13	U1 snRNA single-stranded binding nucleotide sequence annealing from position -2 to +9**	TGGAuGUACCA
14	U1 snRNA single-stranded binding nucleotide sequence annealing from position -1 to +10**	AUGGAuGUACC
15	U1 snRNA single-stranded binding nucleotide sequence annealing from position +1 to +11**	CAUGGAuGUAC
16	U1 snRNA single-stranded binding nucleotide sequence annealing from position +2 to +12**	GCAUGGAuGUA
17	U1 snRNA single-stranded binding nucleotide sequence annealing from position +3 to +13**	GGCAUGGAuGU
18	U1 snRNA single-stranded binding nucleotide sequence annealing from position +4 to +14**	UGGCAUGGAuG
19	U1 snRNA single-stranded binding nucleotide sequence annealing from position +5 to +15**	GUGGCAUGGAu
20	Carrier DNA sequence for encoding U1 snRNA, including cloning site	TAAGGACCAGCTTCTTTGGGAGAGAA CAGACGCAGGGGCGGGAGGGAAAAA GGGAGAGGCAGACGTCACTTCCTCTT GGCGACTCTGGCAGCAGATTGGTCG GTTGAGTGGCAGAAAGGCAGACGGG

		GACTGGGCAAGGCACTGTCGGTGAC ATCACGGACAGGGCGACTTCTATGTA GATGAGGCAGCGCAGAGGCTGCTGC TTCGCCACTTGCTGCTTCGCCACGAA GGGAGTTCCCGTGCCCTGGGAGCGG GTTCAGGACCGCTGATCGGAAGTGA GAATCCCAGCTGTGTGTCAGGGCTG GAAAGGGCTCGGGAGTGCGCGGGG CAAGTGACCGTGTGTGTAAAGAGTGA GGCGTATGAGGCTGTGTCGGGGCAG AGGCACAACGTTTC gagacggaattccgtctc GCAGGGGAGATACCATGATCACGAA GGTGGTTTTCCCAGGGCGAGGCTTAT CCATTGCACTCCGGATGTGCTGACCC CTGCGATTTCCCCAAATGTGGGAAAC TCGACTGCATAATTTGTGGTAGTGGG GGACTGCGTTCGCGCTTTCCCCTG ACTTTCTGGAGTTTCAAAAACAGACT GTACGCCAAGG
21	Promoter region of carrier DNA sequence	TAAGGACCAGCTTCTTTGGGAGAGAA CAGACGCAGGGGCGGGAGGGAAAAA GGGAGAGGCAGACGTCACTTCCTCTT GGCGACTCTGGCAGCAGATTGGTCG GTTGAGTGGCAGAAAGGCAGACGGG GACTGGGCAAGGCACTGTCGGTGAC ATCACGGACAGGGCGACTTCTATGTA GATGAGGCAGCGCAGAGGCTGCTGC TTCGCCACTTGCTGCTTCGCCACGAA GGGAGTTCCCGTGCCCTGGGAGCGG GTTCAGGACCGCTGATCGGAAGTGA GAATCCCAGCTGTGTGTCAGGGCTG GAAAGGGCTCGGGAGTGCGCGGGG CAAGTGACCGTGTGTGTAAAGAGTGA GGCGTATGAGGCTGTGTCGGGGCAG AGGCACAACGTTTC
22	Cloning site of carrier DNA sequence for cloning DNA encoding U1 snRNA single-stranded binding nucleotide sequence	gagacggaattccgtctc
23	Scaffold region of carrier DNA sequence	GCAGGGGAGATACCATGATCACGAA GGTGGTTTTCCCAGGGCGAGGCTTAT CCATTGCACTCCGGATGTGCTGACCC CTGCGATTTCCCCAAATGTGGGAAAC TCGACTGCATAATTTGTGGTAGTGGG GGACTGCGTTCGCGCTTTCCCCTG
24	Terminator region of carrier DNA sequence	ACTTTCTGGAGTTTCAAAAACAGACT GTACGCCAAGG
25	Carrier DNA sequence encoding U1 snRNA, with DNA encoding U1 snRNA single-stranded binding	TAAGGACCAGCTTCTTTGGGAGAGAA CAGACGCAGGGGCGGGAGGGAAAAA GGGAGAGGCAGACGTCACTTCCTCTT

	nucleotide sequence represented as NNNNNNNNNNN	GGCGACTCTGGCAGCAGATTGGTCG GTTGAGTGGCAGAAAGGCAGACGGG GACTGGGCAAGGCACTGTCGGTGAC ATCACGGACAGGGCGACTTCTATGTA GATGAGGCAGCGCAGAGGCTGCTGC TTCGCCACTTGCTGCTTCGCCACGAA GGGAGTTCCCGTGCCCTGGGAGCGG GTTCAGGACCGCTGATCGGAAGTGA GAATCCCAGCTGTGTGTCAGGGCTG GAAAGGGCTCGGGAGTGCGCGGGG CAAGTGACCGTGTGTGTAAAGAGTGA GGCGTATGAGGCTGTGTCGGGGCAG AGGCACAACGTTTC NNNNNNNNNNN GCAGGGGAGATACCATGATCACGAA GGTGGTTTTCCCAGGGCGAGGCTTAT CCATTGCACTCCGGATGTGCTGACCC CTGCGATTTCCCCAAATGTGGGAAAC TCGACTGCATAATTTGTGGTAGTGGG GGACTGCGTTCGCGCTTTCCCCTG ACTTTCTGGAGTTTCAAAAACAGACT GTACGCCAAGG
26	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -13 to -3	TTGGGAGAGGA
27	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -12 to -2	ATTGGGAGAGG
28	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -11 to -1	CATTGGGAGAG
29	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -10 to +1	CCATTGGGAGA
30	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -9 to +2	ACCATTGGGAG
31	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -8 to +3	TACCATTGGGA
32	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -7 to +4	GTACCATTGGG
33	DNA encoding U1 snRNA single-stranded binding nucleotide	tGTACCATTGG

	sequence annealing from position -6 to +5***	
34	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -5 to +6***	AtGTACCATTG
35	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -4 to +7***	GAtGTACCATT
36	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -3 to +8***	GGAtGTACCAT
37	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -2 to +9***	TGGAtGTACCA
38	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position -1 to +10***	ATGGAtGTACC
39	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position +1 to +11***	CATGGAtGTAC
40	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position +2 to +12***	GCATGGAtGTA
41	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position +3 to +13***	GGCATGGAtGT
42	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position +4 to +14***	TGGCATGGAtG
43	DNA encoding U1 snRNA single-stranded binding nucleotide sequence annealing from position +5 to +15***	GTGGCATGGAt
44	FLA-PCR forward primer	CGCTGCTCAGGTTCAACGCCGTGC
45	FLA-PCR reverse primer	GACACACAGCCTGTCCACT
46	qPCR primer 1	CGCTGCTCAGGTTCAACGCCGTGC
47	qPCR primer 2	GCAGAGTGCTCCTCACCAAAC
48	qPCR primer 3	TGGGCTCGGCAATCCAAAC
49	qPCR primer 4	AGGAGCCCATTGTTGATGAA
50	qPCR primer 5	GGTGCCTGGATAAATCTTGGT
51	qPCR forward primer for GAPDH	GCAAATTCCATGGCACCGT
52	qPCR reverse primer for GAPDH	GCCCCACTTGATTTTGGAGG

*In SEQ ID NO: 1, part of the exon sequence is shown in capital letters

**In SEQ ID NOs 9 to 19, the nucleotide “u” in small letters is complementary to the “A” in the c.5714+5G>A mutation

***In SEQ ID NOs 33 to 43, the nucleotide “t” in small letters encodes for the “u” that will be complementary to the “A” in the c.5714+5G>A mutation

Table 1.

[00127] While embodiments of the invention have been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the scope of the invention as defined by the appended claims. The scope of the invention is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

1. A modified U1 small nuclear RNA (snRNA) configured to retain exon 40 in a mature mRNA transcript of a mutated *ABCA4* gene, the mutated *ABCA4* gene comprising a mutation that induces skipping of exon 40, wherein the mutation is located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40, wherein the modification comprises replacing a portion of a single-stranded nucleotide sequence of a 5' region of a wild-type U1 snRNA with a single-stranded binding nucleotide sequence capable of hybridizing to a target sequence present on a pre-mRNA transcript of the mutated *ABCA4* gene, and wherein the target sequence is located in a region between 13 base pairs upstream and 15 base pairs downstream of the donor splice site of exon 40.

2. The modified U1 snRNA of claim 1, wherein the mutation that induces skipping of exon 40 is c.5714+5G>A.

3. The modified U1 snRNA of claims 1 or 2, wherein the region comprises the sequence of SEQ ID NO: 1.

4. The modified U1 snRNA of any one of claims 1 to 3, wherein the single-stranded binding nucleotide sequence is between 11 and 21 nucleotides in length.

5. The modified U1 snRNA of any one of claims 1 to 4, wherein the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 2 to 19.

6. The modified U1 snRNA of any one of claims 1 to 5, wherein the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 7, 8 and 10.

7. A nucleic acid construct comprising a polynucleotide sequence encoding the modified U1 snRNA of any one of claims 1 to 6.

8. The nucleic acid construct of claim 7, wherein the polynucleotide sequence encoding the modified U1 snRNA is operably linked to a RNU1-1 promoter and to a RNU1-1 terminator.

9. The nucleic acid construct of claims 7 or 8, comprising the sequence of SEQ ID NO: 25.

10. The nucleic acid construct of any one of claims 7 to 9, wherein a portion of the polynucleotide sequence that encodes the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 26 to 43.

11. The nucleic acid construct of any one of claims 7 to 10, wherein a portion of the polynucleotide sequence that encodes the single-stranded binding nucleotide sequence comprises a sequence selected from the group consisting of SEQ ID NOs 31, 32 and 34.

12. The nucleic acid construct of any one of claims 7 to 11, wherein the nucleic acid construct is contained in an expression vector.

13. The nucleic acid construct of claim 12, wherein the expression vector is selected from the group consisting of a lentiviral vector and an adeno-associated viral vector.

14. The nucleic acid construct of any one of claims 7 to 11, wherein the nucleic acid construct is contained in a lipid nanoparticle.

15. A modified U1 snRNA according to any one of claims 1 to 6 or a nucleic acid construct according to any one of claims 7 to 14 for use in treating a ABCA4 retinopathy.

16. The modified U1 snRNA according to claim 15 to or the nucleic acid construct of claim 15, wherein the *ABCA4* retinopathy is Stargardt disease.

17. Use of a modified U1 snRNA according to any one of claims 1 to 6 and a nucleic acid construct according to any one of claims 7 to 14 in the manufacture of a medicament for treating a *ABCA4* retinopathy.

18. The use of claim 17, wherein the *ABCA4* retinopathy is Stargardt disease.

19. A method of treating a *ABCA4* retinopathy comprising administering to a subject a composition comprising a modified U1 snRNA according to any one of claims 1 to 6 and a nucleic acid construct according to any one of claims 7 to 14.

20. The method of claim 19, wherein the *ABCA4* retinopathy is Stargardt disease.

21. A pharmaceutical composition comprising (a) a therapeutically effective amount of a modified U1 snRNA according to any one of claims 1 to 6 or a nucleic acid construct according to any one of claims 7 to 14 and (b) one or more pharmaceutically acceptable carriers and/or diluents.

22. A method of retaining exon 40 in a mature mRNA transcript of a mutated *ABCA4* gene, wherein the mutated *ABCA4* gene comprises a mutation that induces skipping of exon 40, wherein the mutation is located between 3 base pairs upstream and 8 base pairs downstream of a donor splice site of exon 40, the method comprising providing a modified U1 snRNA according to any one of claims 1 to 6, wherein binding of the modified U1 snRNA to the target sequence induces the retention of exon 40 in a mature mRNA transcript of the mutated *ABCA4* gene.

23. The method of claim 22, wherein the mutation that induces skipping of exon 40 is c.5714+5G>A.

24. The method of claims 22 or 23, comprising providing a modified U1 snRNA having a single-stranded binding nucleotide sequence that is between 11 and 21 nucleotides in length.

25. The method of any one of claims 22 to 24, comprising providing a modified U1 snRNA having a single-stranded binding nucleotide sequence selected from the group consisting of SEQ ID NOs 2 to 19.

26. The method of any one of claims 22 to 25, comprising providing a modified U1 snRNA having a single-stranded binding nucleotide sequence selected from the group consisting of SEQ ID NOs 7, 8 and 10.