

RECOMBINANT ADENO-ASSOCIATED VIRAL VECTORS FOR TREATING BIETTI CRYSTALLINE DYSTROPHY

FIELD OF THE INVENTION

[001] The present disclosure provides an expression cassette and recombinant adeno-associated virus (rAAV) vector comprising a nucleic acid encoding CYP4V2. Also provided are viral particles comprising a rAAV vector, a composition comprising the viral particles, and uses thereof.

BACKGROUND

[002] Recent progresses have demonstrated that gene therapy holds great promises in treating human genetic disorders[1]. Over 6,000 of inherited diseases have been described[2], the number of which is still increasing with further disclosure of genome sequencing techniques[3]. The discovery of safer delivering vectors and the revelation of new gene editing techniques in recent years significantly enhanced the toolbox for treating these genetic diseases, with which the deficient genes could either be supplemented by exogenous DNAs that carried by a therapeutic vector or be corrected *in situ* permanently by gene editing[4]. Contrary to this promising prospect, the list of genetic disorders that are clinically treatable grows slowly[5]. A major limiting factor to this situation is the lack of human translatable animal models, which impeded the therapeutic effect evaluation of gene therapies[6].

[003] Bietti Crystalline Dystrophy (BCD, MIM 210370) is an autosomal recessive inherited disease first described by the Italian ophthalmologist G.B. Bietti in 1937, which was named for its distinguishing yellow-white crystalline deposits observed in patient's fundus[7]. BCD is accounted for ~3% of all nonsyndromic retinitis pigmentosa (RP) and for ~10% of nonsyndromic autosomal recessive RP in Europe[8]. BCD also appears to be more common in East Asia, especially in China, where the genetic mutation frequency is estimated to be 1 in 20,000[9].

[004] In 2000, it was identified that the genetic basis of BCD is associated with chromosome 4q35[8]. *CYP4V2*, a member of the cytochrome P450 superfamily, was identified as the disease-causing gene of BCD in 2004[10]. Besides the crystalline deposits in retina and cornea, altered fatty acid concentration was also found in the serum of BCD patients, which implied a dysregulation of lipid metabolism[11,12]. More importantly, BCD patients inevitably develop

vision loss and night blindness between 20 and 40 years of age and eventually progress to legal blindness between the ages of 50 and 60[11]. Unfortunately, 80 years after the first discovery of BCD, there is still no treatment available for this severe blinding disease.

[005] AAV has become a preferred delivery tool for gene therapies for treating genetic disorders such as BCD due to its demonstrated long-term transgene expression, selective tropism and non-pathogenic nature.

[006] CN111733174B discloses a construct which can be packaged into AAV vectors and comprises both coding sequences of *CYP4V2* and RdCVF for treating BCD. The construct comprises a CAG promoter and a BGH polyadenylation signal site.

[007] CN109136266A also provides construct for expressing *CYP4V2* by rAAV. In the packaging plasmid, a sequence encoding short peptide C9 is inserted to enable a specific binding to CD59 antigen on human RPE cell membrane. In addition, a sequence encoding HRH peptide is inserted along with the coding sequence of *CYP4V2* to inhibit VEGF.

[008] Both CN111733174B and CN109136266A use the wild type coding sequence of *CYP4V2*.

[009] Accordingly, there is a need in the art for improved gene therapy compositions and methods that can efficiently and safely restore *CYP4V2* gene function in BCD patients.

SUMMARY OF THE INVENTION

[0010] The present inventors have developed rAAV vectors comprising codon-optimized coding sequence of *CYP4V2* and certain combinations of gene expression regulatory sequences, which achieved significantly enhanced expression levels (~26.1 times) as compared to the previously published form[13], thus completing the invention.

[0011] Therefore, in a first aspect, the present disclosure relates to an isolated nucleic acid molecule, comprising a nucleotide sequence selected from a group consisting of SEQ ID NOs: 2-17 and encoding human *CYP4V2*. In a more preferred embodiment, the nucleotide sequence is selected from SEQ ID NOs: 8, 9, 15, 16 and 17. In an even more preferred embodiment, the nucleotide sequence is SEQ ID NO: 8 or SEQ ID NO: 16. In the most preferred embodiment, the nucleotide sequence is SEQ ID NO: 16.

[0012] In an embodiment of the first aspect, the isolated nucleic acid molecule further comprises a promoter which is operatively linked to the 5' of the nucleotide sequence encoding *CYP4V2*.

Preferably, the promoter is CAG promoter of SEQ ID NO: 35.

[0013] In one embodiment, the isolated nucleic acid molecule further comprises a polyadenylation sequence at the 3' of the nucleotide sequence encoding CYP4V2 polypeptide. Preferably, the polyadenylation sequence is bovine growth hormone (bGH) polyA, synthesized polyA (SPA) or Simian Virus 40 (SV40) polyA, more preferably SV40 polyA.

[0014] In another embodiment, the isolated nucleic acid further comprises a Woodchuck Hepatitis Virus posttranscriptional regulatory element (WPRE) located between the *CYP4V2* coding sequence and the SV40 polyA sequence.

[0015] In one preferred embodiment, the isolated nucleic acid molecule comprises

(a) a nucleotide sequence of any one of SEQ ID NOs: 18-34, or

(b) a nucleotide sequence having at least 85% homology, at least 90% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, at least 99% homology to any one of SEQ ID NO: 18-34.

[0016] In a second aspect, the present disclosure provides a recombinant AAV (rAAV) vector comprising the nucleic acid molecule of the first aspect.

[0017] In one embodiment, the rAAV vector comprises at least one ITR, preferably two ITRs. In a preferred embodiment, the two ITRs are derived from AAV2 ITR.

[0018] In a third aspect, the present disclosure provides a viral particle comprising the recombinant AAV vector of the second aspect and AAV capsid selected from AAV1, AAV2, AAV4, AAV5, AAV7, AAV8, AAV9, AAVrh10, AAV2.7m8, AAVAnc80L65 and the variants thereof. Preferably, the capsid is AAV8.

[0019] In a fourth aspect, the present disclosure provides a pharmaceutical composition comprising the viral particle of the third aspect and a pharmaceutically acceptable excipient.

[0020] In a fifth aspect, the present disclosure provides use of the rAAV vector in the manufacture of a medicament for treating or preventing Bietti Crystalline Dystrophy (BCD) or any other diseases of retinal pigment epithelium (RPE) atrophy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] FIG. 1 is an illustrative diagram showing the constructs CMV-*hCyp4v2* (upper panel) and CAG-*hCyp4v2* (BCD1, lower panel).

[0028] FIG. 2 is a histogram showing the *CYP4V2* transgene protein expression levels of the

construct CMV-*hCyp4v2* in comparison with the construct CAG-*hCyp4v2* (BCD1) in HEK293.

[0029] FIG. 3 shows the evaluation of the *CYP4V2* transgene protein expression levels of the constructs BCD1-BCD17 in HEK293 cells.

[0030] FIG. 4 shows the evaluation of the *CYP4V2* transgene protein expression levels of the constructs BCD1-BCD17 in ARPE-19 cells.

[0031] FIG. 5A-B show the *CYP4V2* transgene protein expression levels of the constructs BCD8, BCD9, BCD15, BCD16, and BCD17 in HEK293 cells by western blot (A) and histograms (B).

[0032] FIG. 6A-B show the *CYP4V2* transgene protein expression levels of the constructs BCD8, BCD9, BCD15, BCD16, and BCD17 in ARPE-19 cells by western blot (A) and histograms (B).

[0033] FIG. 7A-B show the *CYP4V2* transgene protein expression levels of the packaged AAV8-BCD1, AAV8-BCD8, and AAV8-BCD16 in the transduced ARPE-19 cells by western blot (A) and histograms (B).

DETAILED DESCRIPTION OF THE INVENTION

[0034] Unless specifically defined elsewhere in this document, all the technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

[0035] As used herein, including the appended claims, the singular forms of words such as “a”, “an”, and “the”, include their corresponding plural references unless the context clearly dictates otherwise.

[0036] In the context of the present disclosure, unless being otherwise indicated, the wording “comprise”, and variations thereof such as “comprises” and “comprising” will be understood to imply the inclusion of a stated element, e.g. an amino acid sequence, a nucleotide sequence, a property, a step or a group thereof, but not the exclusion of any other elements, e.g. amino acid sequences, nucleotide sequences, properties and steps. When used herein the term “comprise” or any variation thereof can be substituted with the term “contain”, “include” or sometimes “have” or equivalent variation thereof. In certain embodiments, the wording “comprise” also include the scenario of “consisting of”.

[0037] The abbreviation “*CYP4V2*” refers to cytochrome P450 family 4 subfamily V member 2, which is a gene coding for CYP4V2 protein of the cytochrome P450 hemethiolate protein superfamily involved in oxidizing various substrates in the metabolic pathway. Defects in the gene *CYP4V2* are known to cause BCD and also fundus dystrophy. The *CYP4V2* mentioned here refers

to human *CYP4V2* in the context of the present disclosure unless otherwise indicated.

Isolated nucleic acid encoding CYP4V2 protein.

[0038] The present disclosure provides an isolated nucleic acid sequence comprising a nucleotide sequence coding for CYP4V2 protein, specifically human CYP4V2. By "**isolated nucleic acid**", it means a DNA or RNA which is removed from all or a portion of a polynucleotide in which the isolated polynucleotide is found in nature, or is linked to a polynucleotide to which it is not linked in nature. An isolated nucleic acid molecule "**comprising**" a specific nucleotide sequence may include, in addition to the specified sequence, operably linked regulatory sequences that control expression of the coding region of the recited nucleic acid sequences. Due to the codon degeneracy, one skilled in the art can understand that a specific amino acid sequence can be coded by different nucleotide sequences.

[0039] The nucleotide sequence coding for CYP4V2 of the present disclosure has been subjected to codon optimization and screening, resulting in an enhanced expression level as compared to the wild-type coding sequence without codon optimization.

[0040] For example, the codon optimized coding sequence of *CYP4V2* can achieve an expression level about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 15-fold, or about 20-fold as compared to the wild-type coding sequence by Western blot analysis of CYP4V2 protein expression levels in HEK293 cells and ARPE-19 cells.

[0041] Preferably, the codon optimized coding sequence of CYP4V2 has a reduced CpG number and/or a reduced number of CpG island. For example, the codon optimized coding sequence comprises less than 100 CpG, less than 70 CpG, less than 20 CpG, less than 15 CpG, less than 10 CpG, less than 5 CpG or 0 CpG. For example, the codon optimized coding sequence does not have CpG islands. By "**CpG island**" it stands for a DNA region of at least 200 bp in length, a GC percentage content greater than 50%, and an observed-to-expected CpG ratio greater than 60%.

[0042] For example, the codon-optimized coding sequence has a percentage sequence identity of lower than 80% as compared to the wild-type coding sequence of CYP4V2 as shown in SEQ ID NO: 1. The percentage identity of two sequences can be calculated by known programs in the art. Due to the different parameters for alignment and different definition of identity, the calculated percentage identity of two sequences may vary depending on the program being used to do the

calculation. In the present disclosure, blastn is used to obtain the percentage identity of two nucleotide sequences, and specifically a “highly similar sequences (megablast)” mode is selected.

[0043] The codon-optimized coding sequence can be selected from a group consisting of the nucleotide sequences as set forth in SEQ ID NOs: 2-17. Preferably, the codon-optimized coding sequence is any one of SEQ ID NOs: 8, 9, 15, 16 and 17. More preferably, the codon-optimized coding sequence is SEQ ID NO: 16.

[0044] The regulatory sequences comprised in the nucleic acid molecule can be selected from one or more of promoter, enhancer, polyadenylation sequence, and translation termination signal. A certain combination of regulatory sequences of the present disclosure can achieve unexpected effect in improving the expression efficiency of the coding sequence.

[0045] The promoter of the present disclosure can be a constitutive promoter, a tissue-specific promoter or a cell type-specific promoter. For example, the promoter can be an RPE cell-specific promoter. The promoter can be the native promoter of *CYP4V2*. In a more preferred embodiment, the promoter is a CAG promoter having a sequence as set forth in SEQ ID NO. 35. CAG promoter is a strong constitutive promoter which drives high levels of gene expression in mammalian expression vectors. CAG promoter consists of a cytomegalovirus (CMV) early enhancer element, a promoter which is picked from the first exon and the first intron of chicken beta-actin gene and a splice acceptor of the rabbit beta-globin gene.

[0046] The Kozak consensus sequence (Kozak sequence), named after the scientist who discovered it, is a nucleic acid motif in most eukaryotic mRNA transcripts that naturally functions as the protein translation initiation site[14]. Kozak sequence ensures the protein is correctly translated and enhances protein expression.

[0047] In addition, the nucleic acid of the present disclosure can further comprise an intron inserted between the promoter and the coding sequence. As known in the art, some introns may enhance the expression of genes in eukaryotes. The intron of the present disclosure can be a part of the naturally occurring intron of the *CYP4V2* gene.

[0048] The polyadenylation sequence of the present disclosure can be bGH polyA, SPA or SV40 polyA [15], preferably SV40 polyA. Anyone of the polyA can be combined with a Woodchuck Hepatitis Virus posttranscriptional regulatory element (WPRE)[16], which is a DNA sequence, when transcribed, creates a tertiary structure enhancing expression, and configured as WPRE-bGH

polyA, WPRE-SPA or WPRE-SV40 poly A, respectively.

[0049] In preferred embodiment of the present disclosure, the nucleic acid sequence comprises a nucleotide sequence encoding for a CAG promoter, a Kozak sequence, a codon-optimized coding sequence of *CYP4V2* gene, and a WPRE-SV40 polyA. For example, the isolated nucleic acid sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 18-34. In a more preferred embodiment, the isolated nucleic acid sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 25, 26, 32, 33, and 34. In the most preferred embodiment, the isolated nucleic acid sequence comprises a nucleotide sequence of SEQ ID NO: 33.

rAAV vectors and viral particles

[0050] The nucleic acid molecule of the present disclosure can be constructed into a recombinant AAV vector, to obtain rAAV particles for delivery into treatment subjects.

[0051] In addition to the inserted nucleotide sequence as described above, the rAAV vectors are in single stranded form. The rAAV vector is usually comprised of two inverted terminal repeat (ITR) sequences at both ends of the inserted nucleotide sequence. The ITR of the present disclosure can be ITR derived from any AAV serotypes. When reference is made to serotype of AAV ITR, the phrase “derived from” means that the ITR can be the ITR of a certain serotype or a variant derived therefrom with modification(s). In a preferred embodiment of the present disclosure, the rAAV vector comprises two ITRs derived from AAV2[17]. For example, the rAAV vector comprises two AAV2 ITRs, or comprises a wild-type AAV2 ITR and an AAV2 ITR variant lacking region C or region C'. For example, the wild-type AAV2 ITR locates at a position at 5' of the inserted nucleotide sequence, while the AAV2 ITR variant locates at a position at 3' of the inserted nucleotide sequence; or *vice versa*.

[0052] The rAAV genome was packaged into an AAV capsid. The capsid can be derived from any AAV serotype known in the art or characterized in the future. The capsid and ITRs can be derived from the same serotype of AAV or from different serotypes of AAV. Preferably, the capsid is suitable for eye delivery, *e.g.*, subretinal, intravitreal or intraocular delivery. In a specific embodiment, the AAV vector comprises a capsid of AAV1, AAV2, AAV4, AAV5, AAV7, AAV8, AAV9, AAVrh10, AAV2.7m8, or AAVAnc80L65 serotype, or a variant thereof.

[0053] In a preferred embodiment of the present disclosure, the rAAV is comprised of AAV

serotype 8 capsids[18]. Evidence have shown that ssAAV8 transduces photoreceptors and retinal pigment epithelial cells (RPE) more efficiently than ssAAV2 and ssAAV5. Under the guidance of the capsid proteins, the virus transduces target cells and then the transferred genome containing *CYP4V2* gene and regulatory elements is released into the targeted RPE. The released rAAV genome remains stable and independent from the host genome to stably produce functional CYP4V2 protein in the treatment subjects.

Pharmaceutical composition

[0001] The term “**pharmaceutical composition**” refers to a composition suitable for delivering to a subject. The pharmaceutical composition of the present disclosure comprises the isolated nucleic acid, the rAAV vector or the viral particle of the present disclosure and a pharmaceutically acceptable excipient. Conventional pharmaceutically acceptable excipients are known in the art and can be solid or liquid excipients.

Therapeutic Uses

[0054] The rAAV, viral particle or composition of the present disclosure can be used to treat or prevent BCD, which is related to *CYP4V2* mutations. In addition, the rAAV, viral particle or composition of the present disclosure may also be effective in treating or preventing other conditions or diseases related to retinal pigment epithelium (RPE) atrophy, such as fundus dystrophy.

[0055] The term “**treat**”, “**treating**” or “**treatment**” includes cure or at least alleviate the symptoms of BCD, or conditions or diseases related to RPE atrophy.

Administration

[0056] The terms “**administration**” and “**administering**” as used herein, when applied to a subject, e.g. an animal, including human, or to cells, tissue, organ, or biological fluid, mean contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the subject, cell, tissue, organ, or biological fluid. The term “**administration**” also includes *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell.

[0057] In the present disclosure, the viral particle or the pharmaceutical composition of the

present application is preferably delivered through intra-ocular delivery, *e.g.*, sub-retinal injection.

EXAMPLES

Example 1. Selection of promoter

[0058] The inventors first prepared a CYP4V2-expression AAV vector comprised of two ITRs, a CMV promoter, a wild type *CYP4V2* CDS and SV40 polyA[13]. Due to the reported silencing risk of CMV promoter[19], the CMV promoter was later replaced with a CAG promoter, resulting in a new vector designated as “*CAG-hCyp4v2* (BCD1)” which showed significantly improved CYP4V2 protein expression (Fig. 1, lower panel).

Example 2. Codon optimization increased *Cyp4v2* expression

[0059] To obtain a transgene construct which could provide higher expression levels of the CYP4V2 protein, the coding sequence of *CYP4V2* was codon optimized to improve codon usage frequency in human cells. In addition, the GC content and CpG islands of the modified coding sequence were reduced to minimize the potential for TLR9-mediated immune responses *in vivo*. Based on the above criteria, 16 different coding sequences (SEQ ID NOs: 2-17) were designed, synthesized and cloned into CAG promoter to obtain 16 more transgene constructs (BCD2-BCD17). Following Table 1 shows the CpG number, CpG island number of SEQ ID NOs: 2-17, as well as their sequence homology to the wild-type sequence.

Table 1. CpG content, CpG island number and sequence identity percentages of 16 codon-optimized sequences in comparison to the wild-type coding sequence

Construct ID	Codon ID	CpG number	CpG islands	Identity%
BCD1	CYP4V2_wt	50	Yes	100
BCD2	CYP4V2_co1	69	Yes	77.15
BCD3	CYP4V2_co2	63	Yes	78.23
BCD4	CYP4V2_co3	62	Yes	76.89
BCD5	CYP4V2_co4	65	Yes	77.34
BCD6	CYP4V2_co5	61	Yes	75.56
BCD7	CYP4V2_co6	0	No	79.25
BCD8	CYP4V2_co7	12	No	78.68
BCD9	CYP4V2_co8	0	No	79.12
BCD10	CYP4V2_co9	0	No	78.99
BCD11	CYP4V2_co10	0	No	79.69
BCD12	CYP4V2_co11	0	No	78.29
BCD13	CYP4V2_co12	0	No	78.8
BCD14	CYP4V2_co13	0	No	79.44
BCD15	CYP4V2_co14	0	No	78.74
BCD16	CYP4V2_co15	0	No	78.74
BCD17	CYP4V2_co16	1	No	76.89

Example 3. Transfection of HEK293 and ARPE-19 cells and detection of expression level

[0060] HEK293 and ARPE-19 cells were maintained in DMEM + 10% FBS and passaged every 3 days by TrypLE. The day before transfection, HEK293 cells were inoculated to a 24-well plate at a density of 1×10^5 cells/cm², while ARPE-19 cells were inoculated to a 24-well plate at a density of 7×10^4 cells /cm². Plasmids were transfected using Lipofectamine 3000 Transfection Reagent (Invitrogen, L3000008) following the user's guide. 72 hours after transfection, cells were collected in RIPA lysis buffer (Beyotime, P0013C) with a protease inhibitor cocktail (Roche, 04693159001) and SDS-PAGE loading buffer (Cowin Bio, CW0027), denatured for 15 min at 95°C, centrifuged at 12,000 rpm for 10 min. Supernatants were separated in 4%-10% SDS-PAGE gel (Cowin Bio, CW0022M), and blotted onto 0.45 μm NC transfer membrane (Merck, HATF00010).

[0061] The protein expression levels of CYP4V2 and the housekeeping gene GAPDH were detected by an antibody against the human CYP4V2 (Sigma, HPA029122) and GAPDH (Abcam, ab8245), respectively. The gray value of bands were calculated and normalized to BCD1.

[0062] The results from repeated experiments showed that transgene constructs BCD8, BCD9, BCD15, BCD16, BCD17 expressed CYP4V2 protein at higher levels among the 16 tested codon optimized vectors as evaluated in HEK293 cells (Figure 3) and ARPE-19 cells (Figure 4).

Accordingly, transgene constructs BCD8, BCD9, BCD15, BCD16 and BCD17 were selected for further evaluation, using BCD1 as a control. Western blot (WB) results are shown in the upper panel of Figure 5 and Figure 6. The gray value of all the bands were calculated, analyzed and normalized to BCD1 (Figure 5 and Figure 6, lower panels).

[0063] Based on the Western blot results, it was determined that BCD8 exhibited the highest CYP4V2 protein expression levels in HEK293 cells (Figure 5) and BCD16 exhibited the highest CYP4V2 protein expression levels in ARPE-19 cells (Figure 6).

[0064] Later, BCD1, BCD8, and BCD16 were packaged to replication-defective AAV8, respectively. AAV8-BCD1, AAV8-BCD8 and AAV8-BCD16 virus particles were used to transduce ARPE-19 at $\text{MOI} = 5 \times 10^5$ in the presence of 2 mM hydroxyurea.

[0065] The AAV transduction experiment showed that AAV8-BCD16 had the highest expression levels (Figure 7).

References

1. Friedmann T, Roblin R. Gene therapy for human genetic disease? *Science*. 1972; 175: 949-955.
2. McKusick VA. Mendelian inheritance in man and its online version, omim. *Am J Hum Genet*. 2007; 80: 588-604.
3. Chen R, Shi LS, Hakenberg J, Naughton B, Sklar P, Zhang JG *et al*. Analysis of 589,306 genomes identifies individuals resilient to severe mendelian childhood diseases. *Nat Biotechnol*. 2016; 34: 531-538.
4. Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. *Science*. 2018; 359.
5. Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR. Gene therapy clinical trials worldwide to 2017: An update. *J Gene Med*. 2018; 20.
6. Casal M, Haskins M. Large animal models and gene therapy. *Eur J Hum Genet*. 2006; 14: 266-272.
7. Bietti GB. Ueber familiaeres vorkommen von "retinitis punctata albescens"(verbunden mit "dystrophia marginalis cristallinea corneae"): Glitzern des glaskoerpers und anderen degenerativen augenveraenderungen. *Klin Mbl Augenheilk*. 1937; 99: 737-756.
8. Jiao XD, Munier FL, Iwata F, Hayakawa M, Kanai A, Lee J *et al*. Genetic linkage of bietti crystallin corneoretinal dystrophy to chromosome 4q35. *Am J Hum Genet*. 2000; 67: 1309-1313.
9. Hu DN. Ophthalmic genetics in china. *Ophthalmic Paed Gen*. 1983; 2: 39-45.
10. Li A, Jiao X, Munier FL, Schorderet DF, Yao W, Iwata F *et al*. Bietti crystalline corneoretinal dystrophy is caused by mutations in the novel gene cyp4v2. *Am J Hum Genet*. 2004; 74: 817-826.
11. Kaiser-Kupfer MI, Chan C-C, Markello TC, Crawford MA, Caruso RC, Csaky KG *et al*. Clinical biochemical and pathologic correlations in bietti's crystalline dystrophy. *American Journal of Ophthalmology*. 1994; 118: 569-582.
12. Lai TYY, Chu KO, Chan KP, Ng TK, Yam GHF, Lam DSC *et al*. Alterations in serum fatty acid

- concentrations and desaturase activities in bietti crystalline dystrophy unaffected by cyp4v2 genotypes. Invest Ophth Vis Sci. 2010; 51: 1092-1097.
13. Qu B, Wu S, Jiao G, Zou X, Li Z, Guo L *et al.* Treating bietti crystalline dystrophy in a high-fat diet-exacerbated murine model using gene therapy. Gene Therapy. 2020.
 14. Kozak M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger rnas. Nucleic acids research. 1987; 15: 8125-8148.
 15. Connelly S, Manley JL. A functional mrna polyadenylation signal is required for transcription termination by rna polymerase ii. Genes & development. 1988; 2: 440-452.
 16. Zufferey R, Donello JE, Trono D, Hope TJ. Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. Journal of virology. 1999; 73: 2886-2892.
 17. Grimm D, Kern A, Rittner K, Kleinschmidt JA. Novel tools for production and purification of recombinant adenoassociated virus vectors. Hum Gene Ther. 1998; 9: 2745-2760.
 18. Gao GP, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99: 11854-11859.
 19. Tenenbaum L, Chtarto A, Lehtonen E, Velu T, Brotchi J, Levivier M. Recombinant aav - mediated gene delivery to the central nervous system. The Journal of Gene Medicine: A cross - disciplinary journal for research on the science of gene transfer and its clinical applications. 2004; 6: S212-S222.

CLAIMS

1. An isolated nucleic acid molecule, comprising a nucleotide sequence selected from a group of sequences consisting of SEQ ID NOs: 2-17 and encoding human CYP4V2 polypeptide.
2. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence is selected from SEQ ID NOs: 8, 9, 15, 16 and 17.
3. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence is SEQ ID NO: 8 or SEQ ID NO: 16.
4. The isolated nucleic acid molecule of any one of claims 1 to 3, further comprising a promoter which is operatively linked to the 5' of the nucleotide sequence encoding CYP4V2.
5. The isolated nucleic acid molecule of claim 4, wherein the promoter is the CAG promoter.
6. The isolated nucleic acid molecule of any one of claims 1 to 5, further comprising a polyadenylation sequence at the 3' of the nucleotide sequence encoding CYP4V2.
7. The isolated nucleic acid molecule of claim 6, wherein the polyadenylation sequence is bovine growth hormone (bGH) polyA, synthesized polyA (SPA) or Simian Virus 40 (SV40) polyA, more preferably SV40 polyA.
8. The isolated nucleic acid molecule of any one of claims 1 to 7, further comprising a Woodchuck Hepatitis Virus posttranscriptional regulatory element (WPRE).
9. The isolated nucleic acid molecule of any one of claims 1 to 8, comprising any of (a) or (b)
 - (a) a nucleotide sequence of any one of SEQ ID NOs: 18-34, or
 - (b) a nucleotide sequence having at least 85% homology, at least 90% homology, at least 95% homology, at least 96% homology, at least 97% homology, at least 98% homology, at least 99% homology to any one of SEQ ID NO: 18-34.
10. A recombinant AAV vector, comprising the nucleic acid molecule of any one of claims 1 to 9.

- 11.** The recombinant AAV vector of claim 10, comprising one or two ITRs.
- 12.** The recombinant AAV vector of claim 11, comprising two AAV2 ITR.
- 13.** A viral particle comprising the recombinant AAV vector of any one of claims 10 to 12 packaged into an AAV capsid.
- 14.** The viral particle of claim 13, wherein the AAV capsid is AAV8 capsid.
- 15.** A pharmaceutical composition comprising the viral particle of claim 13 or claim 14, and a pharmaceutically acceptable excipient.
- 16.** Use of a rAAV vector of any one of claims 10 to 12 in the manufacture of a medicament for treating or preventing Bietti Crystalline Dystrophy (BCD).