CODON-OPTIMISED COMPLEMENT FACTOR I

FIELD OF THE INVENTION

The present invention relates to agents for use in gene therapy. In particular, the invention relates to polynucleotides encoding Complement Factor I (CFI) or Complement Factor H-like Protein 1 (FHL1), vectors comprising said polynucleotides, and their uses in the treatment or prevention of complement-mediated and complement-associated disorders, including eye diseases, such as age-related macular degeneration (AMD).

BACKGROUND TO THE INVENTION

The macula is a small area in the retina of the eye, approximately 3 to 5 millimetres in size, adjacent to the optic nerve. It is the most sensitive area of the retina and contains the fovea, a depressed region that allows for high visual acuity and contains a dense concentration of cones, the photoreceptors that are responsible for colour vision.

Age-related macular degeneration (AMD) is the most common cause of functional blindness in developed countries for persons over 50 years of age (Seddon, J.M., Epidemiology of age-related macular degeneration. In: Ogden, T.E., et al., eds. Ryan S.J., ed-in-chief. Retina Vol II. 3rd ed. St. Louis, Mo.: Mosby; 2001: 1039-1050). AMD is associated with neovascularisation originating from the choroidal vasculature and extending into the subretinal space. In addition, AMD is characterised by progressive degeneration of the retina, retinal pigment epithelium (RPE), and underlying choroid (the highly vascular tissue that lies beneath the RPE, between the retina and the sclera).

A variety of factors including oxidative stress, inflammation with a possible autoimmune component, genetic background (e.g. mutations), and environmental or behavioural factors such as smoking and diet may contribute to the pathogenesis of AMD.

The clinical progression of AMD is characterised in stages according to changes in the macula. The hallmark of early AMD is the appearance of drusen, which are accumulations of extracellular debris underneath the retina and appear as yellow spots in the retina during clinical examination and on fundus photographs. Drusen are categorised by size as small (<63 μm), medium (63-124 μm) and large (>124 μm). They are also considered as hard or soft depending on the appearance of their margins on ophthalmological examination. While hard drusen have clearly defined margins, soft drusen have less defined, fluid margins. The Age-related Eye Disease Study (AREDS) fundus photographic severity scale is one of the main classification systems used for this condition.

AMD has been classified into “dry” and “wet” (exudative or neovascular) forms. Dry AMD is more common than wet AMD, but the dry form can progress to the wet form, and the two occur simultaneously in a significant number of cases. Dry AMD is typically characterised by progressive apoptosis of cells in the RPE layer, overlying photoreceptor cells, and frequently also the underlying cells in the choroidal capillary layer. Confluent areas of RPE cell death accompanied by overlying photoreceptor atrophy are referred to as geographic atrophy. Patients with this form of AMD experience a slow and progressive deterioration in central vision.

Wet AMD is characterised by bleeding and/or leakage of fluid from abnormal vessels that have grown from the choroidal vessels (choriocapillaris) beneath the RPE and the macula, which can be responsible for sudden and disabling loss of vision. It has been estimated that much of the vision loss that patients experience is due to such choroidal neovascularisation (CNV) and its secondary complications. A subtype of neovascular AMD is termed retinal angiomatous proliferation (RAP). Here, angiomatous proliferation originates from the retina and extends posteriorly into the subretinal space, eventually communicating in some cases with choroidal new vessels.

The complement system (CS) has been implicated in early AMD pathogenesis based on the identification of CS components in drusen from eyes of AMD patients. In AMD, at least 129 types of drusen-deposited proteins have been identified, including different apolipoprotein types (E, B or A-I), several amyloid peptides (P, Aβ or SA-1), TIMP-3, serum albumin, and certain proteins associated with cellular function (e.g. ATP synthase β subunit, scavenger receptor B2 and retinol dehydrogenase). AMD-derived drusen also contain almost all of the complement proteins, including regulatory proteins (CFH, complement receptor 1 (CR1), vitronectin and clusterin), the products of CS activation and degradation (C1q, C3, C3a, C3b and C5a), and members of the terminal CS pathway comprising the MAC components (i.e. 5, 6, 8 (α, β and γ) and 9) in the separated and complex form. Accumulating drusen may activate the CS, trigger the local production of inflammatory mediators, and attract leukocytes that in turn augment the local inflammatory state present in AMD.

Current treatment options for AMD include photodynamic therapy with benzoporphyrin (Arch Ophthalmol (1999) 117: 1329-1345) and a number of therapies which target the Vascular Endothelial Growth Factor (VEGF) pathway. Examples of such VEGF-targeted therapies include the aptamer pegaptanib (N Engl J Med (2004) 351: 2805-2816) and antibodies such as ranibizumab (N Engl J Med (2006) 355: 1432-1444) and bevacizumab (BMJ (2010) 340: c2459). However, not all patients respond to treatment with an anti-VEGF antibody and either do not recover vision or progress to registered blindness.

A therapy for the treatment of geographic atrophy has been developed and was used in a phase III clinical study. Lampalizumab is a humanised monoclonal inhibitory antibody to Complement Factor D, administered by intravitreal injection to stop the rate of progression of geographic atrophy. However, in the Phase III randomised clinical trial, involving 906 participants, Lampalizumab failed to reduce GA enlargement when compared with sham over 48 weeks.

Accordingly, there is a significant need in the art for new approaches to treat eye diseases, such as AMD.

Due to the ubiquitous nature of the complement system, overactive or improperly-functioning complement system has been implicated in the pathology of many chronic inflammatory conditions for which treatment options either do not exist or require management of symptoms via regular interventions over a period of years. There is, therefore, a general need to develop gene therapy treatments that provide new or alternative treatments for complement-mediated and complement-associated disorders, particularly chronic inflammatory conditions and even more particularly those which are associated with overactivity of the complement C3b feedback cycle (Figure 1).

SUMMARY OF THE INVENTION

The applicant has identified codon optimised sequences of Complement Factor I (CFI) and Complement Factor H-like Protein 1 (FHL1), which provide for substantially increased expression of the encoded CFI and FHL1 proteins compared to the wild type sequences.

The improved CFI- and FHL1-encoding sequences developed by the applicant enable higher doses of the respective proteins to be delivered to a patient without increasing the amount of vector that is administered. The invention therefore provides improvements in terms of manufacturing output (i.e. protein delivery can be achieved with lower amounts of vector that is produced), efficacy of the medicament and also safety. In particular, as a higher encoded protein dose may be achieved with delivery of the same amount (e.g. volume) of vector, the risk of damage to the tissue to which vector is administered is reduced. For example, when a vector is delivered to the eye by subretinal injection, the risk of damage to or detachment of the retina caused by injection of larger volumes of medicament is reduced. In addition, the risk of off-target effects that arise through spread of larger volumes of medicament to adjacent tissues is reduced. Furthermore, the use of the claimed nucleotide sequences in gene therapy, has the potential to deliver a treatment in a single dose, allowing for long-term, stable expression of protein, and avoiding the need for monthly or regular injections. The nucleotide sequences and compositions of the invention have the additional advantage,

therefore, that they have the potential to provide a one-time or “single-shot” therapy that avoids repeated or regular surgical interventions.

In one aspect, the invention provides an isolated polynucleotide comprising a nucleotide sequence encoding Complement Factor I (CFI), wherein the nucleotide sequence has at least 85% sequence identity to SEQ ID NO: 10.

In some embodiments, the nucleotide sequence encoding CFI has at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 10.

In preferred embodiments, the nucleotide sequence encoding CFI is SEQ ID NO: 10.

In another aspect, the invention provides an isolated polynucleotide comprising a nucleotide sequence encoding Complement Factor H-like Protein 1 (FHL1), wherein the nucleotide sequence has at least 75% sequence identity to SEQ ID NO: 12.

In some embodiments, the nucleotide sequence encoding FHL1 has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 12.

In preferred embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 12.

In some embodiments, the polynucleotide comprises one or more adeno-associated virus (AAV) inverted terminal repeats (ITRs). In preferred embodiments, the polynucleotide comprises an AAV ITR at its 5’ end and an AAV ITR at its 3’ end.

In some embodiments, the AAV ITRs are AAV2 or AAV8 ITRs. In preferred embodiments, the AAV ITRs are AAV2 ITRs.

In another aspect, the invention provides a vector comprising the polynucleotide of the invention.

In some embodiments, the vector is an adeno-associated viral (AAV), retroviral, lentiviral or adenoviral vector.

In preferred embodiments, the vector is an AAV vector.

In some embodiments, the vector is in the form of a viral vector particle.

In some embodiments, the AAV vector particle comprises an AAV2 or AAV8 genome.

In some embodiments, the AAV vector particle comprises AAV2 or AAV8 capsid proteins.

In some embodiments, the AAV vector particle comprises an AAV2 genome and AAV2 capsid proteins (AAV2/2). In other embodiments, the AAV vector particle comprises an AAV2 genome and AAV8 capsid proteins (AAV2/8). In other embodiments, the AAV vector particle comprises an AAV8 genome and AAV8 capsid proteins (AAV8/8).

In some embodiments, the nucleotide sequence encoding the CFI is operably linked to a CMV promoter. In some embodiments, the nucleotide sequence encoding the CFI is operably linked to a regulatory element, such as a WPRE regulatory element. In preferred embodiments, the WPRE regulatory element is a WPRE3 regulatory element. In some embodiments, the nucleotide sequence encoding the CFI is operably linked to a polyadenylation (poly-A) signal, such as a Bovine Growth Hormone poly-A signal.

In preferred embodiments, the nucleotide sequence encoding the CFI is operably linked to a CMV promoter; a WPRE regulatory element (preferably a WPRE3 regulatory element); and a Bovine Growth Hormone poly-A signal.

In some embodiments, the nucleotide sequence encoding the FHL1 is operably linked to a CMV promoter. In some embodiments, the nucleotide sequence encoding the FHL1 is operably linked to a regulatory element, such as WPRE regulatory element. In preferred embodiments, the WPRE regulatory element is a WPRE3 regulatory element. In some embodiments, the nucleotide sequence encoding the FHL1 is operably linked to a poly-A signal, such as a Bovine Growth Hormone poly-A signal.

In preferred embodiments, the nucleotide sequence encoding the FHL1 is operably linked to a CMV promoter; a WPRE regulatory element (preferably a WPRE3 regulatory element); and a Bovine Growth Hormone poly-A signal.

In another aspect, the invention provides a cell comprising the polynucleotide of the invention.

In another aspect, the invention provides a cell transduced with the vector of the invention.

In another aspect, the invention provides a pharmaceutical composition comprising the polynucleotide, vector or cell of the invention in combination with a pharmaceutically acceptable carrier, diluent or excipient.

In a particular embodiment, the pharmaceutical composition is suitable for systemic administration, e.g. by infusion via peripheral vein.

In a particular embodiment, the pharmaceutical composition is suitable for local administration, e.g. intrathecal administration.

In preferred embodiments, the pharmaceutical composition is for intraocular administration for example, by intravitreal injection, suprachoroidal injection or sub-retinal injection.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in therapy.

In a particular embodiment the polynucleotide, vector or cell of the invention is used in the treatment of complement-mediated disorders, particularly chronic inflammatory conditions.

In a preferred embodiment the polynucleotide, vector or cell of the invention is used in the treatment of a disorder associated with overactivity of the complement C3b feedback cycle.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing an ocular disorder.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing a complement-mediated disorder of the eye.

In another aspect, the invention provides a method of treating or preventing a complement-mediated disorder of the eye comprising administering the polynucleotide, vector or cell of the invention to a subject in need thereof.

In another aspect, the invention provides a method of providing Complement Factor I (CFI) and/or Complement Factor H-like Protein 1 (FHL1) to a subject, comprising delivering the polynucleotide, vector or cell of the invention to the eye of the subject.

In some embodiments, the disorder is associated with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle (see Figure 1).

In some embodiments, the disorder is a chronic complement-mediated inflammatory condition of the eye.

In some embodiments, the disorder is age-related macular degeneration (AMD) or diabetic retinopathy. In other embodiments, the disorder is glaucoma, Stargardt's disease, central serous chorioretinopathy or retinitis pigmentosa.

In preferred embodiments, the disorder is AMD. In some embodiments, the AMD is dry AMD.

In some embodiments, a subject has been diagnosed with AMD or is at risk of acquiring AMD.

In some embodiments, the use is for treating or preventing a disorder in a subject:

(a) having lower than normal Complement Factor I activity or concentration in the eye and/or serum, preferably having a concentration of, or activity equivalent to, 0-30, 0-20 or 0-10 µg/mL in serum; and/or

(b) being heterozygous or homozygous for an age-related macular degeneration (AMD)-associated SNP, preferably a rare Complement Factor I variant.

In some embodiments, the use is for treating or preventing a disorder in a subject:

(a) having a normal level of Complement Factor I activity or concentration in the eye and/or serum, preferably at least 30 µg/mL, such as 30-40 µg/mL in serum; and/or

(b) not carrying a rare Complement Factor I variant allele.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing age-related macular degeneration (AMD). In preferred embodiments, the AMD is dry AMD.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in treating or preventing diabetic retinopathy.

In some embodiments, the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.

In some embodiments, the progression of geographic atrophy is slowed.

In some embodiments, there is at least a 10% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period. In other embodiments, there is at least 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period

In some embodiments, administration of the polynucleotide, vector or cell increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the

retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in improving or restoring vision or visual acuity, for example in a subject suffering from an eye disorder, such as an eye disorder disclosed herein. In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in mitigating loss of vision or visual acuity, for example a loss of vision or visual acuity associated with an eye disorder, such as an eye disorder disclosed herein.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in improving or restoring reading speed in a subject, for example in a subject suffering from an eye disorder, such as an eye disorder disclosed herein. In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in mitigating reduction in reading speed in a subject, for example a reduction in reading speed associated with an eye disorder, such as an eye disorder disclosed herein.

In another aspect, the invention provides the polynucleotide, vector or cell of the invention for use in reducing or preventing loss of photoreceptors and/or the retinal pigment epithelium (RPE), for example a loss of photoreceptors and/or the RPE associated with an eye disorder, such as an eye disorder disclosed herein.

In some embodiments, the polynucleotide, vector or cell is administered intraocularly.

In some embodiments, the polynucleotide, vector or cell is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

In some embodiments, the polynucleotide, vector or cell is administered to the eye of a subject by subretinal injection.

In some embodiments, the polynucleotide or vector of the invention does not comprise a hAAT promoter. In some embodiments, the polynucleotide or vector of the invention does not comprise an ApoR enhancer. In other embodiments, the polynucleotide or vector of the invention does not comprise two ApoR enhancers.

In some embodiments, the vector of the invention does not comprise an AAV2 genome and an AAV8 capsid protein, i.e. the vector of the invention is not an AAV2/8 vector.

In some embodiments, the polynucleotide, vector or cell of the invention is not administered systemically. In other embodiments, the polynucleotide, vector or cell of the invention is not administered intravenously.

DESCRIPTION OF THE DRAWINGS

FIGURE 1

C3b feedback (amplification) and breakdown (down-regulation) cycles of the alternative pathway of vertebrate complement ("I" = Complement Factor I; "H" = Complement Factor H; "B" = Complement Factor B; and "D" = Complement Factor D).

FIGURE 2

Western blot analyses of supernatants from codon-optimised CFI and FHL1 plasmid transfection of ARPE19 cells.

FIGURE 3

ELISA analyses of supernatants from codon-optimised CFI plasmid transfection of ARPE19 cells.

FIGURE 4

ELISA analyses of supernatants from codon-optimised FHL1 plasmid transfection of ARPE19 cells.

FIGURE 5

ELISA analyses of supernatants from codon-optimised CFI AAV vector transduction of ARPE19 cells.

FIGURE 6

ELISA analyses of supernatants from codon-optimised FHL1 AAV vector transduction of ARPE19 cells.

DETAILED DESCRIPTION OF THE INVENTION

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including" or "includes"; or "containing" or "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements or steps. The terms "comprising", "comprises" and "comprised of" also include the term "consisting of".

COMPLEMENT SYSTEM

The complement system is an integral part of the humoral immune system and is involved in tissue inflammation, cell opsonisation, and cytolysis. It provides protection against microorganisms and mediates the clearance of exogenous and endogenous cellular debris from the host tissues.

The complement system cascade is comprised of four activation pathways. All of the pathways ultimately end in the central cleavage of C3 factor and in the generation of its active fragments C3a and C3b. C3a is the anaphylatoxin that triggers a range of chemotactic and proinflammatory responses, such as recruitment of inflammatory cells and increased microvasculature permeability, whereas C3b is responsible for opsonisation of foreign surfaces covalently attached to C3b. Opsonisation with activated C3 fragments (C3b and iC3b) fulfils three major functions: (i) cell debris elimination by phagocytic cells (e.g. macrophages or microglia) and the stimulation of the adaptive immune system (B and T cells); (ii) amplification of complement activation via the formation of a surface-bound C3 convertase; and (iii) assemblage of the C5 convertase.

Assemblage of the C5 convertase is responsible for C5 cleavage, which results in the formation of the cytolytic membrane attack complex (MAC) capable of generating perforations in the cell membrane, thereby promoting cell lysis and the elimination of unnecessary cells. Through all of these activities, the innate complement cascade supports and promotes the function of downstream mechanisms of the immune system that protect the integrity of the host tissue. Overall, complement system pathway activation results in a proinflammatory response, including MAC generation, which mediates cell lysis, the release of chemokines to attract inflammatory cells to the site of damage, and the enhancement of capillary permeability to promote extravasation of infiltrating leukocytes. Under physiological conditions, complement activation is effectively controlled by the coordinated action of soluble and membrane-associated complement regulatory molecules (CRMs). Soluble complement regulators, such as C1-inhibitor, anaphylatoxins inhibitor, C4b binding protein (C4BP), Complement Factor H (CFH), Complement Factor I (CFI), clusterin and vitronectin, restrict the action of complement in human tissues at multiple sites of the cascade reaction. In addition, each individual cell is protected against the attack of homologous complement by surface proteins, such as the Complement Receptor 1 (CR1, CD35), the membrane cofactor protein (CD46), and glycosylphosphatidylinositol-anchored proteins, such as decay-accelerating factor (CD55) or CD59 molecule. Of note, host cells and tissues that are inadequately protected from complement attack might be subjected to bystander cell lysis.

The invention relates to the treatment or prevention of a complement-mediated disorder of the eye. For example, the complement-mediated disorder may be a disorder associated with a defect in alternative pathway regulation, and in particular with over-activity of the complement C3b feedback cycle and/or under-activity of the C3b breakdown cycle.

In some embodiments, prior to administration of the polynucleotide, vector, cell or pharmaceutical composition of the invention, the subject has low levels (e.g. lower than normal levels) of Complement Factor I activity, for example low levels of Complement Factor I activity in the eye and/or low serum levels of Complement Factor I activity. The sub-normal level of Complement Factor I activity may be due to sub-normal expression of normally-functioning Complement Factor I, or at least partial (e.g. heterozygous) expression (at normal or sub-normal levels) of a non- or sub-functional variant of Complement Factor I. (Such a subject may carry one or more copies of an AMD-associated SNP, for example the subject may be homo- or heterozygous for one of the rare Complement Factor I variants discussed further below). Thus, the subject may have a low concentration (e.g. a lower than normal concentration) of Complement Factor I in the eye and/or serum. For a human subject, the normal level of Complement Factor I activity (C3b-inactivating and iC3b-degradation activity) may be equivalent to that provided by 30-40 µg/mL Complement Factor I in the serum of the subject. Thus, in a subject with low Complement Factor I activity, the Complement Factor I activity in the serum may correspond to less than 30 µg/mL and greater than 0 µg/mL Complement Factor I, such as 0-20 or 0-10 µg/mL (these being ranges of Complement Factor I serum concentration which may encompass a subject having a low Complement Factor I concentration).

Thus, the subject to be treated by the invention may suffer from a complement-mediated disorder of the eye such as AMD, more particularly dry AMD (e.g. characterised by geographic atrophy), or may be at risk of developing such a disorder. For example, the subject may be homozygous or heterozygous susceptible for one or more SNPs associated with the complement-mediated disorder.

In some embodiments, the subject is at risk of developing AMD. For example, the subject may be homozygous or heterozygous susceptible for one or more SNPs associated with AMD, for example rare mutations in Complement Factor I associated with advanced AMD which commonly result in reduced serum Complement Factor I levels (Kavanagh et al. (2015) Hum Mol Genet 24: 3861-3870). In particular the subject may carry one or two copies of one or more of the following rare Complement Factor I variants: rs144082872 (encoding P50A); 4:110687847 (encoding P64L); rs141853578 (encoding G119R); 4:110685721 (encoding V152M); 4:110682846 (encoding G162D); 4:110682801 (encoding N177I);

rs146444258 (encoding A240G); rs182078921 (encoding G287R); rs41278047 (encoding K441R); and rs121964913 (encoding R474).

The invention may further comprise determining whether the subject is at risk of developing a complement-mediated disorder (for example, AMD), for example by determining whether the subject is homozygous or heterozygous susceptible for one or more SNPs associated with the complement-mediated disorder (for example, by determining whether the subject is homozygous or heterozygous susceptible for one or more of the rare Complement Factor I variants associated with AMD listed above).

Alternatively, the subject may have a normal level of endogenous Complement Factor I activity or concentration, for example in the eye and/or serum and/or may not carry a rare variant Complement Factor I allele.

In some embodiments, administration of the polynucleotide, vector, cell or pharmaceutical composition of the invention thereby increases the level of C3b-inactivating and iC3b-degradation activity in the eye of the subject. In other embodiments, administration of the polynucleotide, vector, cell or pharmaceutical composition of the invention thereby increases the level of C3b-inactivating and iC3b-degradation activity in the eye of the subject to a level that exceeds a normal level in the eye. More particularly, the level of C3b-inactivating and iC3b-degradation activity is increased in the RPE of the eye.

It will be appreciated that the C3b-inactivating and iC3b-degradation activity in the subject following expression of the Complement Factor I from the polynucleotide or vector of the invention may comprise C3b-inactivating and iC3b-degradation activity from the subject's endogenous Complement Factor I (i.e. the subject's Complement Factor I not produced by expression from the polynucleotide or vector), and C3b-inactivating and iC3b-degradation activity produced by expression from the polynucleotide or vector of the invention, such that the total level of C3b-inactivating and iC3b-degradation activity in the subject exceeds a normal level.

In some embodiments, the level of C3b-inactivating and iC3b-degradation activity in the subject, for example in the eye, is increased to a level that is at least 5%, 10%, 15%, 20% or 25% above the normal level.

In other embodiments, the level of C3b-inactivating and iC3b-degradation activity in the subject, for example in the eye, is increased to a level that is up to twice the normal level, or up to 80%, 60%, 40% or 20% above the normal level.

For example, the level of C3b-inactivating and iC3b-degradation activity in the subject, for example in the eye, may be increased to a level that is 5-100%, 5-80%, 5-60%, 5-40%, 5-20%, 10-100%, 10-80%, 10-60%, 10-40%, 10-20%, 15-100%, 15-80%, 15-60%, 15-40%, 15-20%, 20-100%, 20-80%, 20-60%, 20-40%, 25-100%, 25-80%, 25-60% or 25-40% above the normal level.

In some embodiments, administration of the polynucleotide, vector, cell or pharmaceutical composition of the invention does not detectably increase the level of C3b-inactivating and iC3b-degradation activity in the plasma/serum of the subject. In other embodiments, administration of the polynucleotide, vector, cell or pharmaceutical composition of the invention does not detectably increase the level of C3b-inactivating and iC3b-degradation activity in the plasma/serum of the subject to a level greater than the normal level.

In the foregoing section, except where obviously inapplicable, reference to Complement Factor I and C3b-inactivating and iC3b-degradation activity may be replaced with Complement Factor H or Complement Factor H-like Protein 1, and ability to act as a cofactor for the Complement Factor I mediated cleavage of C3b and to increase the rate of dissociation of C3 convertase and C5 convertase, respectively. In some embodiments, prior to administration of the polynucleotide, vector, cell or pharmaceutical composition of the invention, the subject has low levels (e.g. lower than normal levels) of Complement Factor H, for example low levels of Complement Factor H in the eye and/or low serum levels of Complement Factor H. For a human subject, the normal level of Complement Factor H may be about 200-500 µg/mL in the serum of the subject. Thus, in a subject with low levels of Complement Factor H, the levels in the serum may be less than 200 µg/mL and greater than 0 µg/mL, such as 0-100 µg/mL. Alternatively, the subject may have a normal level of endogenous Complement Factor H, for example in the eye and/or serum.

COMPLEMENT FACTOR I (CFI)

Complement Factor I (Factor I, CFI), also known as C3b/C4b inactivator, is a protein that in humans is encoded by the *CFI* gene.

Complement Factor I is a serine protease that circulates in a zymogen-like state (Roversi et al. (2011) PNAS 108: 12839-12844) at a concentration of ~35 µg/mL (Nilsson et al. (2011) Mol Immunol 48: 1611-1620). The Complement Factor I protein is a heavily N-glycosylated heterodimer consisting of two polypeptide chains linked by a single disulfide bond. The heavy chain (50 kDa) comprises an N-terminal region; an FI membrane attack complex (FIMAC) domain; a CD5 like-domain or scavenger receptor cysteine-rich (SRCR) domain; two low-density lipoprotein receptor (LDLr) domains; and a C-terminal region of unknown

function that is a site of sequence variability across species (Roversi et al. (2011) PNAS 108: 12839-12844). The light chain (38 kDa) contains the serine protease (SP) domain with the conserved catalytic residues (Goldberger et al. (1987) J Biol Chem 262: 10065-10071).

Complement Factor I inactivates C3b by cleaving it into iC3b, C3d and C3d,g and, in an analogous way, C4b into C4c and C4d. To properly perform its functions, Complement Factor I requires the presence of cofactor proteins such as C4b-Binding Protein (C4BP), Complement Factor H (CFH), Complement Receptor 1 (CR1/CD35) and Membrane Cofactor Protein (MCP/CD46) (Degn et al. (2011) Am J Hum Genet 88: 689-705).

iC3b is incapable of associating with Factor B, and thus cannot perpetuate amplification of the complement cascade or activation through the alternative pathway. Hence, once C3b has been cleaved to iC3b, neither alternative pathway initiation nor terminal complement cascade activation occurs.

iC3b is capable of providing a proinflammatory action by binding to, and activating, Complement Receptor 3 (CR3)(CD11b/CD18) on polymorphonuclear leukocytes (mostly neutrophils), NK cells and mononuclear phagocytes, such as macrophages.

Complement Factor I is capable of processing iC3b into C3d,g via a protease activity requiring the cofactor, CR1. C3d,g is unable to bind to CR3. Since iC3b reacting with the complement receptor CR3 is a major mechanism by which complement activation gives rise to inflammation, the breakdown of iC3b to C3d,g is essential for reducing complement-induced inflammation (Lachmann (2009) Adv. Immunol. 104: 115-149).

Complement Factor I's unique ability to both promote cleavage of C3b to iC3b as well as accelerate breakdown of iC3b – combined with its relatively low concentration in human serum, with implications for the amount required to be delivered for therapeutic efficacy – make it a particularly advantageous target.

In some embodiments, a Complement Factor I polypeptide is capable of cleaving C3b into an inactive degradation product. For example, the Complement Factor I polypeptide may be capable of cleaving C3b into iC3b.

In some embodiments, a Complement Factor I polypeptide is capable of processing iC3b into an inactive degradation product. For example, the Complement Factor I polypeptide may be capable of processing iC3b into C3d,g.

In preferred embodiments, the Complement Factor I polypeptide is capable of cleaving C3b into iC3b and processing iC3b into C3d,g.

Suitably, a fragment or derivative of Complement Factor I may retain at least 50%, 60%, 70%, 80%, 90%, 95% or 100% of the C3b-inactivating and iC3b-degradation activity of native Complement Factor I.

The C3b-inactivating and iC3b-degradation activity of Complement Factor I, or a fragment or derivative thereof, may be determined using any suitable method known to the skilled person. For example, measurement of Complement Factor I proteolytic activity is described in Hsiung et al. (Biochem. J. (1982) 203: 293-298). Both haemolytic and conglutinating assays for CFI activity are described in Lachmann PJ & Hobart MJ (1978) "Complement Technology" in Handbook of Experimental Immunology 3rd edition Ed DM Weir Blackwells Scientific Publications Chapter 5A p17. A more detailed description, also including a proteolytic assay, is given by Harrison RA (1996) in "Weir's Handbook of Experimental Immunology" 5th Edition Eds; Herzenberg Leonore A'Weir DM, Herzenberg Leonard A & Blackwell C Blackwells Scientific Publications Chapter 75 36-37. The conglutinating assay is highly sensitive and can be used for detecting both the first (double) clip converting fixed C3b to iC3b and acquiring reactivity with conglutinin; and for detecting the final clip to C3dg by starting with fixed iC3b and looking for the loss of reactivity with conglutinin. The haemolytic assay is used for the conversion of C3b to iC3b, and the proteolytic assay detects all the clips.

In some embodiments, the Complement Factor I is human Complement Factor I.

An example human Complement Factor I protein is the human Complement Factor I protein having the UniProtKB accession number P05156. This exemplified sequence is 583 amino acids in length (disclosed as SEQ ID NO: 1) of which amino acids 1 to 18 form a signal sequence.

In some embodiments, the amino acid sequence of Complement Factor I is SEQ ID NO: 1. In other embodiments, the amino acid sequence of Complement Factor I is the sequence disclosed as positions 19 to 583 of SEQ ID NO: 1.

MKLLHVFLLFLCFHLRFCKVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGT
AVCATNRRSFPTYCQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVDQDKTMFICKSS
WSMREANVACLDLGFQQGADTQRRFKLSDLSINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT
QKADSPMDDFFQCVNGKYISQMKACDGINDCGDQSDELCCKACQGKGFHCKSGVCIPSQYQCNGEVDCITGE
DEVGCAGFASVTQEETEILTADMDAERRRIKSLLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVAIKDA
SGITCGGIYIGGCWILTAAHCLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIAL
IEMKKDGNKKDCELPRSIPACVPWSPYLFQPNDTCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFY
EKEMECAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRP
FISQYNV

(SEQ ID NO: 1)

In some embodiments, the amino acid sequence of Complement Factor I is SEQ ID NO: 9, which corresponds to NCBI Accession No. NP_000195. In other embodiments, the amino acid sequence of Complement Factor I is the sequence disclosed as positions 19 to 583 of SEQ ID NO: 9.

MKLLHVFLLFLCFHLRFCKVTYTSQEDLVEKKCLAKKYTHLSCDKVFCQPWQRCIEGTCVCKLPYQCPKNGT
AVCATNRRSFPTYCQQKSLECLHPGTKFLNNGTCTAEGKFSVSLKHGNTDSEGIVEVKLVDQDKTMFICKSS
WSMREANVACLDLGFQQGADTQRRFKLSDLSINSTECLHVHCRGLETSLAECTFTKRRTMGYQDFADVVCYT
QKADSPMDDFFQCVNGKYISQMKACDGINDCGDQSDELCCKACQGKGFHCKSGVCIPSQYQCNGEVDCITGE
DEVGCAGFASVAQEETEILTADMDAERRRIKSLLPKLSCGVKNRMHIRRKRIVGGKRAQLGDLPWQVAIKDA
SGITCGGIYIGGCWILTAAHCLRASKTHRYQIWTTVVDWIHPDLKRIVIEYVDRIIFHENYNAGTYQNDIAL
IEMKKDGNKKDCELPRSIPACVPWSPYLFQPNDTCIVSGWGREKDNERVFSLQWGEVKLISNCSKFYGNRFY
EKEMECAGTYDGSIDACKGDSGGPLVCMDANNVTYVWGVVSWGENCGKPEFPGVYTKVANYFDWISYHVGRP
FISQYNV

(SEQ ID NO: 9)

An example wild type nucleotide sequence encoding Complement Factor I is the nucleotide sequence having the NCBI Accession No. NM_000204, disclosed herein as SEQ ID NO: 2.

ATGAAGCTTCTTCATGTTTTCCTGTTATTTCTGTGCTTCCACTTAAGGTTTTGCAAGGTCACTTATACATCT
CAAGAGGATCTGGTGGAGAAAAAGTGCTTAGCAAAAAAATATACTCACCTCTCCTGCGATAAAGTCTTCTGC
CAGCCATGGCAGAGATGCATTGAGGGCACCTGTGTTTGTAAACTACCGTATCAGTGCCCAAAGAATGGCACT
GCAGTGTGTGCAACTAACAGGAGAAGCTTCCCAACATACTGTCAACAAAAGAGTTTGGAATGTCTTCATCCA
GGGACAAAGTTTTTAAATAACGGAACATGCACAGCCGAAGGAAAGTTTAGTGTTTCCTTGAAGCATGGAAAT
ACAGATTCAGAGGGAATAGTTGAAGTAAAACTTGTGGACCAAGATAAGACAATGTTCATATGCAAAAGCAGC
TGGAGCATGAGGGAAGCCAACGTGGCCTGCCTTGACCTTGGGTTTCAACAAGGTGCTGATACTCAAAGAAGG
TTTAAGTTGTCTGATCTCTCTATAAATTCCACTGAATGTCTACATGTGCATTGCCGAGGATTAGAGACCAGT
TTGGCTGAATGTACTTTTACTAAGAGAAGAACTATGGGTTACCAGGATTTCGCTGATGTGGTTTGTTATACA
CAGAAAGCAGATTCTCCAATGGATGACTTCTTTCAGTGTGTGAATGGGAAATACATTTCTCAGATGAAAGCC
TGTGATGGTATCAATGATTGTGGAGACCAAAGTGATGAACTGTGTTGTAAAGCATGCCAAGGCAAAGGCTTC
CATTGCAAATCGGGTGTTTGCATTCCAAGCCAGTATCAATGCAATGGTGAGGTGGACTGCATTACAGGGGAA
GATGAAGTTGGCTGTGCAGGCTTTGCATCTGTGGCTCAAGAAGAAACAGAAATTTTGACTGCTGACATGGAT
GCAGAAAGAAGACGGATAAAATCATTATTACCTAAACTATCTTGTGGAGTTAAAAACAGAATGCACATTCGA
AGGAAACGAATTGTGGGAGGAAAGCGAGCACAACTGGGAGACCTCCCATGGCAGGTGGCAATTAAGGATGCC
AGTGGAATCACCTGTGGGGGAATTTATATTGGTGGCTGTTGGATTCTGACTGCTGCACATTGTCTCAGAGCC
AGTAAAACTCATCGTTACCAAATATGGACAACAGTAGTAGACTGGATACACCCCGACCTTAAACGTATAGTA
ATTGAATACGTGGATAGAATTATTTTCCATGAAAACTACAATGCAGGCACTTACCAAAATGACATCGCTTTG
ATTGAAATGAAAAAAGACGGAAACAAAAAAGATTGTGAGCTGCCTCGTTCCATCCCTGCCTGTGTCCCCTGG
TCTCCTTACCTATTCCAACCTAATGATACATGCATCGTTTCTGGCTGGGGACGAGAAAAAGATAACGAAAGA
GTCTTTTCACTTCAGTGGGGTGAAGTTAAACTAATAAGCAACTGCTCTAAGTTTTACGGAAATCGTTTCTAT
GAAAAAGAAATGGAATGTGCAGGTACATATGATGGTTCCATCGATGCCTGTAAAGGGGACTCTGGAGGCCCC
TTAGTCTGTATGGATGCCAACAATGTGACTTATGTCTGGGGTGTTGTGAGTTGGGGGGAAAACTGTGGAAAA
CCAGAGTTCCCAGGTGTTTACACCAAAGTGGCCAATTATTTTGACTGGATTAGCTACCATGTAGGAAGGCCT
TTTATTTCTCAGTACAATGTATAA

(SEQ ID NO: 2)

The nucleotide sequences of Complement Factor I used in the invention are preferably codon-optimised. Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression. By the same

token, it is possible to decrease expression by deliberately choosing codons for which the corresponding tRNAs are known to be rare in the particular cell type. Thus, an additional degree of translational control is available.

A preferred nucleotide sequence encoding Complement Factor I is the nucleotide sequence disclosed as SEQ ID NO: 10.

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ATGAAACTGCTGCATGTCTTCCTCCTCTTCCTGTGCTTCCACCTCCGTTTCTGTAAAGTCACCTACACTAGC
CAGGAGGATCTGGTGGAGAAGAAATGCCTGGCCAAGAAGTATACCCACCTGAGCTGCGACAAAGTGTTCTGC
CAGCCCTGGCAACGCTGCATTGAAGGTACTTGTGTGTGCAAGCTGCCCTACCAGTGCCCCAAGAACGGCACG
GCCGTGTGTGCCACCAACAGGAGGAGCTTCCCCACCTACTGCCAGCAGAAGAGCCTGGAATGCCTCCACCCT
GGCACCAAGTTTCTGAACAACGGGACCTGCACAGCCGAGGGGAAATTCAGCGTCTCCCTCAAGCACGGCAAT
ACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATGTTCATCTGCAAAAGCAGC
TGGTCCATGCGGGAGGCCAATGTCGCCTGCCTGGACCTGGGCTTCCAGCAGGGCGCTGATACACAGCGCCGC
TTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCACGTGCACTGCCGGGGCCTGGAGACCAGC
CTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATGGGCTACCAGGATTTTGCAGATGTGGTCTGCTACACC
CAGAAGGCAGACAGCCCCATGGATGACTTCTTCCAGTGTGTCAATGGCAAGTACATTTCCCAGATGAAGGCT
TGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTCTGCTGCAAGGCCTGCCAAGGGAAGGGCTTT
CACTGTAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCAACGGAGAGGTGGACTGCATCACTGGGGAG
GACGAGGTGGGCTGTGCTGGCTTCGCCTCTGTGGCCCAGGAGGAGACAGAGATCCTCACAGCTGACATGGAT
GCAGAGCGGCGGCGCATCAAGAGTCTGCTCCCAAAGCTCTCCTGCGGCGTTAAGAATCGCATGCACATCCGG
AGGAAGCGGATCGTTGGAGGCAAACGGGCTCAGCTGGGGGACTTGCCGTGGCAGGTGGCCATCAAAGATGCC
TCCGGAATCACCTGTGGTGGCATCTACATCGGCGGCTGCTGGATCCTGACCGCCGCCCACTGCCTTCGGGCC
AGCAAGACTCACCGCTACCAGATCTGGACCACCGTGGTGGATTGGATTCACCCCGACCTGAAGAGGATTGTC
ATTGAGTATGTCGACCGCATCATCTTCCATGAAAACTACAATGCCGGGACGTATCAGAACGACATCGCCCTC
ATCGAGATGAAGAAGGATGGGAACAAGAAGGACTGTGAGCTGCCTCGCTCCATCCCCGCCTGTGTACCATGG
TCTCCGTACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGG
GTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTTCTACGGCAACCGCTTCTAT
GAGAAGGAGATGGAGTGCGCCGGCACCTATGACGGCAGCATTGACGCGTGCAAGGGAGACAGTGGGGGCCCC
CTGGTCTGCATGGACGCCAACAATGTGACCTACGTGTGGGGAGTTGTGTCCTGGGGCGAGAACTGTGGCAAG
CCTGAGTTCCCGGGCGTGTACACAAAGGTGGCAAACTATTTTGACTGGATCTCCTATCACGTTGGCAGGCCC
TTCATTTCACAGTACAACGTATAA
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(SEQ ID NO: 10)

In some embodiments, the nucleotide sequence encoding Complement Factor I has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 10. Preferably, the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 1 or 9.

In other embodiments, the nucleotide sequence encoding Complement Factor I is SEQ ID NO: 10.

In other embodiments, the nucleotide sequence encoding Complement Factor I has at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to positions 55 to 1752 of SEQ ID NO: 10. Preferably, the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 1 or 9.

In other embodiments, the nucleotide sequence encoding Complement Factor I is positions 55 to 1752 of SEQ ID NO: 10.

A further example codon-optimised nucleotide sequence encoding Complement Factor I is SEQ ID NO: 8.

ATGAAGCTGCTGCATGTCTTTCTGCTGTTTCTGTGCTTCCATCTGCGGTTCTGTAAAGTGACCTATACTAGC
CAGGAGGATCTGGTGGAGAAGAAGTGTCTGGCCAAGAAGTACACACACCTGAGCTGCGACAAGGTGTTCTGT
CAGCCTTGGCAGCGGTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCTTACCAGTGCCCAAAGAACGGCACC
GCCGTGTGCGCCACAAATCGGAGATCTTTTCCAACATATTGCCAGCAGAAGAGCCTGGAGTGTCTGCACCCC
GGCACCAAGTTCCTGAACAATGGCACCTGCACAGCCGAGGGCAAGTTTTCTGTGAGCCTGAAGCACGGCAAC
ACAGATAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGATAAGACCATGTTCATCTGTAAGAGCTCC
TGGTCCATGAGGGAGGCAAACGTGGCATGCCTGGATCTGGGATTCCAGCAGGGAGCAGACACACAGAGGCGC
TTTAAGCTGTCCGACCTGTCTATCAATAGCACCGAGTGCCTGCACGTGCACTGTAGGGGCCTGGAGACATCC
CTGGCAGAGTGCACCTTCACAAAGCGGAGAACCATGGGCTACCAGGACTTTGCCGACGTGGTGTGCTATACC
CAGAAGGCCGATAGCCCCATGGACGATTTCTTTCAGTGCGTGAACGGCAAGTATATCTCCCAGATGAAGGCC
TGCGACGGCATCAATGACTGTGGCGATCAGTCTGACGAGCTGTGCTGTAAGGCCTGTCAGGGCAAGGGCTTC
CACTGCAAGAGCGGCGTGTGCATCCCTTCCCAGTACCAGTGCAACGGCGAGGTGGATTGTATCACAGGAGAG
GACGAAGTGGGATGCGCAGGATTTGCATCTGTGGCACAGGAGGAGACAGAGATCCTGACAGCCGACATGGAT
GCCGAGAGGCGCCGGATCAAGTCTCTGCTGCCTAAGCTGAGCTGTGGCGTGAAGAATCGGATGCACATCAGA
AGGAAGCGCATCGTGGGAGGCAAGAGGGCACAGCTGGGCGATCTGCCATGGCAGGTGGCCATCAAGGACGCC
TCTGGCATCACCTGCGGCGGCATCTACATCGGAGGATGTTGGATCCTGACCGCAGCACACTGCCTGAGAGCA
AGCAAGACACACAGGTATCAGATCTGGACCACAGTGGTGGATTGGATCCACCCAGACCTGAAGAGAATCGTG
ATCGAGTACGTGGATAGGATCATCTTTCACGAGAACTACAATGCCGGCACATATCAGAACGACATCGCCCTG
ATCGAGATGAAGAAGGATGGCAATAAGAAGGACTGTGAGCTGCCCAGATCCATCCCTGCATGCGTGCCATGG
AGCCCCTATCTGTTCCAGCCCAACGATACCTGCATCGTGTCCGGATGGGGAAGGGAGAAGGACAATGAGCGG
GTGTTTTCTCTGCAGTGGGGCGAGGTGAAGCTGATCTCCAACTGTTCTAAGTTCTACGGCAATAGGTTTTAT
GAGAAGGAGATGGAGTGCGCCGGCACCTACGATGGCAGCATCGACGCCTGTAAGGGCGATTCCGGAGGACCA
CTGGTGTGCATGGACGCAAACAATGTGACATACGTGTGGGGAGTGGTGTCCTGGGGAGAGAACTGCGGCAAG
CCAGAGTTCCCCGGCGTATATACCAAGGTGGCCAATTATTTTGATTGGATTTCCTACCACGTCGGCAGGCCC
TTTATTTCCCAGTATAATGTCTAA

(SEQ ID NO: 8)

An advantage of the invention is that Complement Factor I is particularly difficult to prepare in the form of a purified protein. Accordingly, the inventors have devised a way of modulating the complement system, for example to enable treatments of age-related macular degeneration (AMD), by administering Complement Factor I in the form of an AAV vector comprising a Complement Factor I-encoding nucleotide sequence. The AAV vector may be administered to a site of interest, for example the eye, to enable in situ translation of the Complement Factor I polypeptide.

COMPLEMENT FACTOR H (CFH)

Complement Factor H (Factor H, CFH) is a complement control protein.

Complement Factor H is a large (155 kDa), soluble glycoprotein that is present in human plasma at a typical concentration of 200–300 µg/mL (Hakobyan et al. (2008) 49(5): 1983-90). The principal function of Complement Factor H is to regulate the alternative pathway of the complement system.

Complement Factor H provides cofactor activity for the Complement Factor I-mediated cleavage of C3b. Complement Factor H also increases the rate of dissociation of the C3bBb

complex (C3 convertase) and the (C3b)NBB complex (C5 convertase) and thereby reduces the activity of the alternative complement pathway.

Complement Factor H is made up of 20 complement control protein (CCP) modules (also referred to as Short Consensus Repeats or sushi domains) connected to one another by short linkers (of between three and eight amino acid residues) and arranged in an extended head to tail fashion. Each of the CCP modules consists of around 60 amino acids with four cysteine residues disulfide bonded in a 1-3 2-4 arrangement, and a hydrophobic core built around an almost invariant tryptophan residue. The CCP modules are numbered from 1-20 (from the N-terminus of the protein). CCPs 1-4 and CCPs 19-20 engage with C3b while CCPs 7 and CCPs 19-20 bind to GAGs and sialic acid (Schmidt et al. (2008) Journal of Immunology 181: 2610–2619).

It has been shown that gene therapy using Complement Factor H can ameliorate induced AMD-like pathology in mice (Cashman et al. (2015) J. Gene Med. 17: 229-243). Mice were co-injected subretinally with: (i) an adenoviral vector expressing complement component C3, which had previously been shown to recapitulate many pathological features of human AMD; and (ii) an adenoviral vector expressing Complement Factor H. Relative to control animals receiving GFP instead of Complement Factor H, the Complement Factor H-transduced mice showed 91% reduction in endothelial cell proliferation and 69% attenuation of RPE atrophy. Electroretinography showed improved retinal function in mice receiving Complement Factor H, and immunocytochemistry of rhodopsin and RPE65 was consistent with the rescue of photoreceptors and RPE in such animals.

In some embodiments, a Complement Factor H polypeptide or a fragment or derivative thereof is capable of acting as a cofactor for the Complement Factor I-mediated cleavage of C3b. In some embodiments, a Complement Factor H polypeptide or a fragment or derivative thereof is capable of increasing the rate of dissociation of C3 convertase and C5 convertase.

In preferred embodiments, a Complement Factor H polypeptide or a fragment or derivative thereof is capable of acting as a cofactor for the Complement Factor I-mediated cleavage of C3b and increasing the rate of dissociation of C3 convertase and C5 convertase.

In some embodiments, the Complement Factor H is human Complement Factor H.

An example human Complement Factor H protein is the human Complement Factor H protein having the UniProtKB accession number P08603. This exemplified sequence is 1231 amino acids in length (disclosed as SEQ ID NO: 3) of which amino acids 1 to 18 form a signal sequence.

In some embodiments, the amino acid sequence of Complement Factor H is SEQ ID NO: 3. In other embodiments, the amino acid sequence of Complement Factor H is positions 19 to 1231 of SEQ ID NO: 3.

MRLLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNVIMVCRKGEWV
ALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVV
KCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVI
NGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGD
EITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYYCDEH
FETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCM
ENGWSPTPRCIRVKTCSKSSIDIENGFISESQYTYALKEKAKYQCKLGYVTADGETSGSITCGKDGWSAQPT
CIKSCDIPVFMNARTKNDFTWFKLNDTLDYECHDGYESNTGSTTGSIVCGYNGWSDLPICYERECELPKIDV
HLVPDRKKDQYKVGEVLKFSCKPGFTIVGPNSVQCYHFGLSPDLPICKEQVQSCGPPPELLNGNVKEKTKEE
YGHSEVVEYYCNPRFLMKGPNKIQCVDGEWTTLPVCIVEESTCGDIPELEHGWAQLSSPPYYYGDSVEFNCS
ESFTMIGHRSITCIHGVWTQLPQCVAIDKLKKCKSSNLIILEEHLKNKKEFDHNSNIRYRCRGKEGWIHTVC
INGRWDPEVNCSMAQIQLCPPPPQIPNSHNMTTTLNYRDGEKVSVLCQENYLIQEGEEITCKDGRWQSIPLC
VEKIPCSQPPQIEHGTINSSRSSQESYAHGTKLSYTCEGGFRISEENETTCYMGKWSSPPQCEGLPCKSPPE
ISHGVVAHMSDSYQYGEEVTYKCFEGFGIDGPAIAKCLGEKWSHPPSCIKTDCLSLPSFENAIPMGEKKDVY
KAGEQVTYTCATYYKMDGASNVTCINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQCRSP
YEMFGDEEVMCLNGNWTEPPQCKDSTGKCGPPPPIDNGDITSFPLSVYAPASSVEYQCQNLYQLEGNKRITC
RNGQWSEPPKCLHPCVISREIMENYNIALRWTAKQKLYSRTGESVEFVCKRGYRLSSRSHTLRTTCWDGKLE
YPTCAKR

(SEQ ID NO: 3)

An example nucleotide sequence encoding Complement Factor H is the nucleotide sequence having the NCBI Accession No. NM_000186.

In some embodiments, the nucleotide sequence encoding Complement Factor H is SEQ ID NO: 4.

ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGTAGCAGAAGATTGCAATGAACTT
CCTCCAAGAAGAAATACAGAAATTCTGACAGGTTCCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCT
ATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAAATGTAATAATGGTATGCAGGAAGGGAGAATGGGTT
GCTCTTAATCCATTAAGGAAATGTCAGAAAAGGCCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTT
ACCCTTACAGGAGGAAATGTGTTTGAATATGGTGTAAAAGCTGTGTATACATGTAATGAGGGGTATCAATTG
CTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
AAGTGTTTACCAGTGACAGCACCAGAGAATGGAAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGAATAC
CATTTTGGACAAGCAGTACGGTTTGTATGTAACTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCATTGT
TCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAAATTTCATGCAAATCCCCAGATGTTATA
AATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTCAATATAAATGTAACATGGGT
TATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAA
AAATCATGTGATAATCCTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAACTGGAGAT
GAAATCACGTACCAGTGTAGAAATGGTTTTTATCCTGCAACCCGGGGAAATACAGCAAAATGCACAAGTACT
GGCTGGATACCTGCTCCGAGATGTACCTTGAAACCTTGTGATTATCCAGACATTAAACATGGAGGTCTATAT
CATGAGAATATGCGTAGACCATACTTTCCAGTAGCTGTAGGAAAATATTACTCCTATTACTGTGATGAACAT
TTTGAGACTCCGTCAGGAAGTTACTGGGATCACATTCATTGCACACAAGATGGATGGTCGCCAGCAGTACCA
TGCCTCAGAAAATGTTATTTTCCTTATTTGGAAAATGGATATAATCAAAATCATGGAAGAAAGTTTGTACAG
GGTAAATCTATAGACGTTGCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATG
GAGAATGGCTGGTCTCCTACTCCCAGATGCATCCGTGTCAAAACATGTTCCAAATCAAGTATAGATATTGAG
AATGGGTTTATTTCTGAATCTCAGTATACATATGCCTTAAAAGAAAAAGCGAAATATCAATGCAAACTAGGA
TATGTAACAGCAGATGGTGAAACATCAGGATCAATTACATGTGGGAAAGATGGATGGTCAGCTCAACCCACG
TGCATTAAATCTTGTGATATCCCAGTATTTATGAATGCCAGAACTAAAAATGACTTCACATGGTTTAAGCTG
AATGACACATTGGACTATGAATGCCATGATGGTTATGAAAGCAATACTGGAAGCACCACTGGTTCCATAGTG
TGTGGTTACAATGGTTGGTCTGATTTACCCATATGTTATGAAAGAGAATGCGAACTTCCTAAAATAGATGTA
CACTTAGTTCCTGATCGCAAGAAAGACCAGTATAAAGTTGGAGAGGTGTTGAAATTCTCCTGCAAACCAGGA

TTTACAATAGTTGGACCTAATTCCGTTCAGTGCTACCACTTTGGATTGTCTCCTGACCTCCCAATATGTAAA
GAGCAAGTACAATCATGTGGTCCACCTCCTGAACTCCTCAATGGGAATGTTAAGGAAAAAACGAAAGAAGAA
TATGGACACAGTGAAGTGGTGGAATATTATTGCAATCCTAGATTTCTAATGAAGGGACCTAATAAAATTCAA
TGTGTTGATGGAGAGTGGACAACTTTACCAGTGTGTATTGTGGAGGAGAGTACCTGTGGAGATATACCTGAA
CTTGAACATGGCTGGGCCCAGCTTTCTTCCCCTCCTTATTACTATGGAGATTCAGTGGAATTCAATTGCTCA
GAATCATTTACAATGATTGGACACAGATCAATTACGTGTATTCATGGAGTATGGACCCAACTTCCCCAGTGT
GTGGCAATAGATAAACTTAAGAAGTGCAAATCATCAAATTTAATTATACTTGAGGAACATTTAAAAAACAAG
AAGGAATTCGATCATAATTCTAACATAAGGTACAGATGTAGAGGAAAAGAAGGATGGATACACACAGTCTGC
ATAAATGGAAGATGGGATCCAGAAGTGAACTGCTCAATGGCACAAATACAATTATGCCCACCTCCACCTCAG
ATTCCCAATTCTCACAATATGACAACCACACTGAATTATCGGGATGGAGAAAAAGTATCTGTTCTTTGCCAA
GAAAATTATCTAATTCAGGAAGGAGAAGAAATTACATGCAAAGATGGAAGATGGCAGTCAATACCACTCTGT
GTTGAAAAAATTCCATGTTCACAACCACCTCAGATAGAACACGGAACCATTAATTCATCCAGGTCTTCACAA
GAAAGTTATGCACATGGGACTAAATTGAGTTATACTTGTGAGGGTGGTTTCAGGATATCTGAAGAAAATGAA
ACAACATGCTACATGGGAAAATGGAGTTCTCCACCTCAGTGTGAAGGCCTTCCTTGTAAATCTCCACCTGAG
ATTTCTCATGGTGTTGTAGCTCACATGTCAGACAGTTATCAGTATGGAGAAGAAGTTACGTACAAATGTTTT
GAAGGTTTTGGAATTGATGGGCCTGCAATTGCAAAATGCTTAGGAGAAAAATGGTCTCACCCTCCATCATGC
ATAAAAACAGATTGTCTCAGTTTACCTAGCTTTGAAAATGCCATACCCATGGGAGAGAAGAAGGATGTGTAT
AAGGCGGGTGAGCAAGTGACTTACACTTGTGCAACATATTACAAAATGGATGGAGCCAGTAATGTAACATGC
ATTAATAGCAGATGGACAGGAAGGCCAACATGCAGAGACACCTCCTGTGTGAATCCGCCCACAGTACAAAAT
GCTTATATAGTGTCGAGACAGATGAGTAAATATCCATCTGGTGAGAGAGTACGTTATCAATGTAGGAGCCCT
TATGAAATGTTTGGGGATGAAGAAGTGATGTGTTTAAATGGAAACTGGACGGAACCACCTCAATGCAAAGAT
TCTACAGGAAAATGTGGGCCCCCTCCACCTATTGACAATGGGGACATTACTTCATTCCCGTTGTCAGTATAT
GCTCCAGCTTCATCAGTTGAGTACCAATGCCAGAACTTGTATCAACTTGAGGGTAACAAGCGAATAACATGT
AGAAATGGACAATGGTCAGAACCACCAAAATGCTTACATCCGTGTGTAATATCCCGAGAAATTATGGAAAAT
TATAACATAGCATTAAGGTGGACAGCCAAACAGAAGCTTTATTCGAGAACAGGTGAATCAGTTGAATTTGTG
TGTAAACGGGGATATCGTCTTTCATCACGTTCTCACACATTGCGAACAACATGTTGGGATGGGAAACTGGAG
TATCCAACTTGTGCAAAAAGATAG

(SEQ ID NO: 4)

In some embodiments, the nucleotide sequence encoding Complement Factor H has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 4. Preferably, wherein the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H is SEQ ID NO: 4.

In other embodiments, the nucleotide sequence encoding Complement Factor H has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to positions 55 to 3696 of SEQ ID NO: 4. Preferably, wherein the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H is positions 55 to 3696 of SEQ ID NO: 4.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes an amino acid sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 3. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes the amino acid sequence SEQ ID NO: 3.

In other embodiment, the nucleotide sequence encoding Complement Factor H encodes an amino acid sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to positions 19 to 1231 of SEQ ID NO: 3. Preferably, wherein the amino acid sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 3.

In other embodiments, the nucleotide sequence encoding Complement Factor H encodes the amino acid sequence of positions 19 to 1231 of SEQ ID NO: 3.

COMPLEMENT FACTOR H-LIKE PROTEIN 1 (FHL1)

Complement Factor H-like Protein 1 (FHL1) is a splice variant of Complement Factor H that contains the first 7 CCPs of Complement Factor H followed by a four amino acid carboxy-terminal tail (Clark, S.J. et al. (2015) J Clin Med 4: 18-31).

In some embodiments, the FHL1 is human FHL1.

In some embodiments, the amino acid sequence of FHL1 is SEQ ID NO: 11.

```
MRLLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRSLGNIIMVCRKGEWV
ALNPLRKCQKRPCGHPGDTPFGTFTLTGGNVFEYGVKAVYTCNEGYQLLGEINYRECDTDGWTNDIPICEVV
KCLPVTAPENGKIVSSAMEPDREYHFGQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVI
NGSPISQKIIYKENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHRTGD
EITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRRPYFPVAVGKYYSYYCDEH
FETPSGSYWDHIHCTQDGWSPAVPCLRKCYFPYLENGYNQNYGRKFVQGKSIDVACHPGYALPKAQTTVTCM
ENGWSPTPRCIRVSFTL

(SEQ ID NO: 11)
```

The nucleotide sequences of FHL1 used in the invention are preferably codon optimised.

A preferred nucleotide sequence encoding FHL1 is SEQ ID NO: 12.

```
ATGCGCCTCCTGGCCAAGATCATCTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTG
CCGCCCAGGAGGAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCACCCAGGCG
ATCTACAAGTGCCGGCCGGGCTACAGGAGCCTGGGGAACATCATCATGGTGTGTAGAAAGGGCGAATGGGTG
GCCCTCAACCCCCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCCTTCGGGACCTTC
ACCCTGACCGGCGGCAATGTGTTTGAGTACGGCGTGAAGGCTGTCTACACATGCAACGAGGGGTACCAGCTG
CTGGGCGAGATTAACTACCGGGAGTGTGACACCGATGGGTGGACCAACGACATTCCCATCTGTGAGGTGGTC
AAGTGTCTCCCCGTGACAGCCCCAGAAAATGGCAAAATCGTGAGCAGCGCCATGGAGCCTGACCGCGAATAT
CACTTTGGGCAGGCCGTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAGATGCACTGC
AGCGATGATGGCTTCTGGTCCAAGGAGAAGCCCAAATGTGTGGAGATCTCCTGCAAGTCTCCCGACGTGATC
AACGGCAGCCCAATCAGCCAGAAGATTATTTACAAAGAGAACGAGCGCTTCCAGTACAAGTGTAACATGGGC
TATGAGTATTCAGAGAGGGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGCTGCGAGGAA
AAGAGTTGTGACAACCCTTACATCCCAAATGGCGACTACTCCCCTCTGCGGATCAAACACCGGACCGGGGAT
GAAATCACCTATCAGTGCCGCAATGGATTCTACCCGGCCACCCGCGGCAACACCGCCAAATGCACCAGCACA
```

GGCTGGATCCCCGCCCCCCGCTGTACGCTGAAGCCTTGCGACTATCCAGACATCAAGCACGGAGGCCTGTAC
CACGAAAACATGCGGCGGCCTTATTTCCCTGTGGCAGTGGGGAAGTACTACAGCTACTACTGCGACGAGCAC
TTCGAGACCCCCTCTGGCTCCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTGCCC
TGCCTGAGGAAATGCTACTTCCCCTACCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTCGTGCAG
GGCAAGAGCATCGATGTGGCCTGCCACCCTGGCTACGCCCTGCCCAAGGCCCAGACAACTGTGACCTGCATG
GAGAATGGTTGGAGCCCCACCCCGCGCTGCATCCGGGTGTCCTTCACGCTCTGA

(SEQ ID NO: 12)

In some embodiments, the nucleotide sequence encoding FHL1 has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 12. Preferably, the protein encoded by the nucleotide sequence substantially retains a functional activity of the protein represented by SEQ ID NO: 11.

In other embodiments, the nucleotide sequence encoding FHL1 is SEQ ID NO: 12.

POLYNUCLEOTIDE

Polynucleotides of the invention may comprise DNA or RNA, preferably DNA. They may be single-stranded or double-stranded. It will be understood by a skilled person that numerous different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides of the invention to reflect the codon usage of any particular host organism in which the polypeptides of the invention are to be expressed.

The polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the in vivo activity or lifespan of the polynucleotides of the invention.

Polynucleotides such as DNA polynucleotides may be produced recombinantly, synthetically or by any means available to those of skill in the art. They may also be cloned by standard techniques.

Longer polynucleotides will generally be produced using recombinant means, for example using polymerase chain reaction (PCR) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking the target sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture with an agarose gel) and recovering the amplified DNA. The primers may

be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable vector.

STRUCTURE OF THE EYE

The medicaments disclosed herein may be delivered to a mammalian, preferably human eye in relation to the treatment or prevention of an eye disease, such as age-related macular degeneration (AMD).

The person skilled in the treatment of diseases of the eye will have a detailed and thorough understanding of the structure of the eye. However, the following structures of particular relevance to the invention are described.

Retina

The retina is the multi-layered membrane, which lines the inner posterior chamber of the eye and senses an image of the visual world which is communicated to the brain via the optic nerve. In order from the inside to the outside of the eye, the retina comprises the layers of the neurosensory retina and retinal pigment epithelium, with the choroid lying outside the retinal pigment epithelium.

Neurosensory retina and photoreceptor cells

The neurosensory retina harbours the photoreceptor cells that directly sense light. It comprises the following layers: internal limiting membrane (ILM); nerve fibre layer; ganglion cell layer; inner plexiform layer; inner nuclear layer; outer plexiform layer; outer nuclear layer (nuclei of the photoreceptors); external limiting membrane (ELM); and photoreceptors (inner and outer segments of the rods and cones).

The skilled person will have a detailed understanding of photoreceptor cells. Briefly, photoreceptor cells are specialised neurons located in the retina that convert light into biological signals. Photoreceptor cells comprise rod and cone cells, which are distributed differently across the retina.

Rod cells are distributed mainly across the outer parts of the retina. They are highly sensitive and provide for vision at low light levels. There are on average about 125 million rod cells in a normal human retina.

Cone cells are found across the retina, but are particularly highly concentrated in the fovea, a pit in the neurosensory retina that is responsible for central high resolution vision. Cone

cells are less sensitive than rod cells. There are on average about 6-7 million cone cells in a normal human retina.

Retinal pigment epithelium

The retinal pigment epithelium (RPE) is a pigmented layer of cells located immediately to the outside of the neurosensory retina. The RPE performs a number of functions, including transport of nutrients and other substances to the photoreceptor cells, and absorption of scattered light to improve vision.

Choroid

The choroid is the vascular layer situated between the RPE and the outer sclera of the eye. The vasculature of the choroid enables provision of oxygen and nutrients to the retina.

AGE-RELATED MACULAR DEGENERATION (AMD)

The clinical progression of age-related macular degeneration (AMD) is characterised in stages according to changes in the macula. The hallmark of early AMD is the appearance of drusen, which are accumulations of extracellular debris underneath the retina and appear as yellow spots in the retina during clinical examination and on fundus photographs. Drusen are categorised by size as small (<63 μm), medium (63-124 μm) and large (>124μm). They are also considered as hard or soft depending on the appearance of their margins on opthalmological examination. While hard drusen have clearly defined margins, soft drusen have less defined, fluid margins. The Age-related Eye Disease Study (AREDS) fundus photographic severity scale is one of the main classification systems used for this condition.

AMD is classified into “dry” and “wet” (exudative or neovascular) forms. Dry AMD is more common than wet AMD, but the dry form can progress to the wet form, and the two occur simultaneously in a significant number of cases. Dry AMD is typically characterised by progressive apoptosis of cells in the RPE layer, overlying photoreceptor cells, and frequently also the underlying cells in the choroidal capillary layer. Confluent areas of RPE cell death accompanied by overlying photoreceptor atrophy are referred to as geographic atrophy (GA). Patients with this form of AMD experience a slow and progressive deterioration in central vision.

Wet AMD is characterised by bleeding and/or leakage of fluid from abnormal vessels that have grown from the choroidal vessels (choriocapillaris) beneath the RPE and the macula, which can be responsible for sudden and disabling loss of vision. It has been estimated that

much of the vision loss that patients experience is due to such choroidal neovascularisation (CNV) and its secondary complications.

The treatment or prevention of AMD described herein may reduce or prevent the appearance of an AMD phenotype described above. Preferably, the treatment of AMD enables maintenance or improvement in visual function.

In some embodiments, the treatment or prevention of AMD results in a prevention of or reduction in the formation of geographic atrophy. In other embodiments, the treatment or prevention of AMD results in slowing the progression of geographic atrophy. For example, it results in an at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% reduction in the increase in GA area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period. In other embodiments, the treatment or prevention of AMD results in the treatment of geographic atrophy, for example a reduction in the amount of geographic atrophy.

In some embodiments, the treatment or prevention of AMD results in a prevention of or reduction in the formation of drusen. In other embodiments, the treatment or prevention of AMD results in a reduction in existing drusen, for example a reduction in the size and/or number of existing drusen.

In some embodiments, the treatment or prevention of AMD results in a prevention of or reduction in complement deposition. In other embodiments, the treatment or prevention of AMD results in a reduction in existing complement deposition.

In some embodiments, the treatment or prevention of AMD results in an improvement in or restoration of vision or visual acuity. In other embodiments, the treatment or prevention of AMD mitigates the loss of vision or visual acuity.

In some embodiments, the treatment or prevention of AMD results in an improvement in or restoration of reading speed in a subject. In other embodiments, the treatment or prevention of AMD mitigates the reduction in reading speed in a subject.

In some embodiments, the treatment or prevention of AMD results in a reduction or prevention of loss of photoreceptors and/or the retinal pigment epithelium (RPE).

DIABETIC RETINOPATHY

Diabetic retinopathy is a condition characterised by damage to the blood vessels of the retina, which is caused by the high blood sugar levels associated with diabetes. If left untreated, diabetic retinopathy can cause blindness.

Although subjects with mild diabetic retinopathy may have good vision, two types of diabetic retinopathy, namely diabetic macular oedema (DMO) and proliferative diabetic retinopathy (PDR) may threaten the sight of the subject.

Diabetic macular oedema is characterised by the leakage of fluid from the damaged blood vessels in the back of the eye. The leaked fluid accumulates in the macula, which leads to swelling and blurred vision. This can eventually give rise to poor central vision and an inability to read or drive. Side vision usually remains normal.

Proliferative diabetic retinopathy is characterised by the closure of retinal blood vessels, leading to the growth of abnormal, fragile blood vessels on the surface of the retina. This may result in permanent loss of vision due to bleeding into the eye, scarring and retinal detachment.

VECTORS

A vector is a tool that allows or facilitates the transfer of an entity from one environment to another.

Adeno-associated viral (AAV) vectors

In one aspect, the invention provides an AAV vector comprising a polynucleotide of the invention.

Preferably, the AAV vector is in the form of an AAV vector particle.

Methods of preparing and modifying viral vectors and viral vector particles, such as those derived from AAV, are well known in the art.

The AAV vector may comprise an AAV genome or a fragment or derivative thereof.

AAV is known to be capable of packaging genomes up to 5.2 kb in size (Dong, J.-Y. et al. (1996) Human Gene Therapy 7: 2101-2112).

An AAV genome is a polynucleotide sequence, which may encode functions needed for production of an AAV particle. These functions include those operating in the replication and packaging cycle of AAV in a host cell, including encapsidation of the AAV genome into an AAV particle. Naturally occurring AAVs are replication-deficient and rely on the provision of

helper functions in trans for completion of a replication and packaging cycle. Accordingly, the AAV genome of the AAV vector of the invention is typically replication-deficient.

The AAV genome may be in single-stranded form, either positive or negative-sense, or alternatively in double-stranded form. The use of a double-stranded form allows bypass of the DNA replication step in the target cell and so can accelerate transgene expression.

The AAV genome may be from any naturally derived serotype, isolate or clade of AAV. Thus, the AAV genome may be the full genome of a naturally occurring AAV. As is known to the skilled person, AAVs occurring in nature may be classified according to various biological systems.

Commonly, AAVs are referred to in terms of their serotype. A serotype corresponds to a variant subspecies of AAV which, owing to its profile of expression of capsid surface antigens, has a distinctive reactivity which can be used to distinguish it from other variant subspecies. Typically, a virus having a particular AAV serotype does not efficiently cross-react with neutralising antibodies specific for any other AAV serotype.

AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 and AAV11, and also recombinant serotypes, such as Rec2 and Rec3, recently identified from primate brain. Any of these AAV serotypes may be used in the invention.

In some embodiments, the AAV vector particle is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rec2 or Rec3 AAV vector particle.

In some embodiments, the AAV may be an AAV1, AAV2, AAV5, AAV7 or AAV8 serotype.

In some embodiments, the AAV may be an AAV2 or AAV8 serotype.

In some embodiments, the AAV may be an AAV2 serotype. In other embodiments, the AAV may be an AAV8 serotype.

The capsid protein may be a mutant capsid protein such as disclosed in WO 2008/124724, which is hereby incorporated by reference.

In some embodiments, the AAV vector comprises an AAV8 capsid with an Y733F mutation.

Reviews of AAV serotypes may be found in Choi et al. (2005) Curr. Gene Ther. 5: 299-310 and Wu et al. (2006) Molecular Therapy 14: 316-27. The sequences of AAV genomes or of elements of AAV genomes including ITR sequences, rep or cap genes for use in the invention may be derived from the following accession numbers for AAV whole genome

sequences: Adeno-associated virus 1 NC_002077, AF063497; Adeno-associated virus 2 NC_001401; Adeno-associated virus 3 NC_001729; Adeno-associated virus 3B NC_001863; Adeno-associated virus 4 NC_001829; Adeno-associated virus 5 Y18065, AF085716; Adeno-associated virus 6 NC_001862; Avian AAV ATCC VR-865 AY186198, AY629583, NC_004828; Avian AAV strain DA-1 NC_006263, AY629583; Bovine AAV NC_005889, AY388617.

AAV may also be referred to in terms of clades or clones. This refers to the phylogenetic relationship of naturally derived AAVs, and typically to a phylogenetic group of AAVs which can be traced back to a common ancestor, and includes all descendants thereof. Additionally, AAVs may be referred to in terms of a specific isolate, i.e. a genetic isolate of a specific AAV found in nature. The term genetic isolate describes a population of AAVs which has undergone limited genetic mixing with other naturally occurring AAVs, thereby defining a recognisably distinct population at a genetic level.

The skilled person can select an appropriate serotype, clade, clone or isolate of AAV for use in the invention on the basis of their common general knowledge. For instance, the AAV5 capsid has been shown to transduce primate cone photoreceptors efficiently as evidenced by the successful correction of an inherited colour vision defect (Mancuso et al. (2009) Nature 461: 784-7).

The AAV serotype determines the tissue specificity of infection (or tropism) of an AAV. Accordingly, preferred AAV serotypes for use in AAVs administered to patients in accordance with the invention are those which have natural tropism for or a high efficiency of infection of target cells within the eye. In some embodiments, AAV serotypes for use in the invention are those which transduce cells of the neurosensory retina, retinal pigment epithelium and/or choroid.

Typically, the AAV genome of a naturally derived serotype, isolate or clade of AAV comprises at least one inverted terminal repeat sequence (ITR). An ITR sequence acts in cis to provide a functional origin of replication and allows for integration and excision of the vector from the genome of a cell. In preferred embodiments, one or more ITR sequences flank the nucleotide sequences encoding the Complement Factor I or FHL1. The AAV genome typically also comprises packaging genes, such as rep and/or cap genes which encode packaging functions for an AAV particle. The rep gene encodes one or more of the proteins Rep78, Rep68, Rep52 and Rep40 or variants thereof. The cap gene encodes one or more capsid proteins such as VP1, VP2 and VP3 or variants thereof. These proteins make up the capsid of an AAV particle. Capsid variants are discussed below.

A promoter will be operably linked to each of the packaging genes. Specific examples of such promoters include the p5, p19 and p40 promoters (Laughlin et al. (1979) Proc. Natl. Acad. Sci. USA 76: 5567-5571). For example, the p5 and p19 promoters are generally used to express the rep gene, while the p40 promoter is generally used to express the cap gene.

As discussed above, the AAV genome used in the AAV vector of the invention may therefore be the full genome of a naturally occurring AAV. For example, a vector comprising a full AAV genome may be used to prepare an AAV vector or vector particle in vitro. However, while such a vector may in principle be administered to patients, this will rarely be done in practice. Preferably, the AAV genome will be derivatised for the purpose of administration to patients. Such derivatisation is standard in the art and the invention encompasses the use of any known derivative of an AAV genome, and derivatives which could be generated by applying techniques known in the art. Derivatisation of the AAV genome and of the AAV capsid are reviewed in Coura and Nardi (2007) Virology Journal 4: 99, and in Choi et al. and Wu et al., referenced above.

Derivatives of an AAV genome include any truncated or modified forms of an AAV genome which allow for expression of a transgene from an AAV vector of the invention in vivo. Typically, it is possible to truncate the AAV genome significantly to include minimal viral sequence yet retain the above function. This is preferred for safety reasons to reduce the risk of recombination of the vector with wild-type virus, and also to avoid triggering a cellular immune response by the presence of viral gene proteins in the target cell.

Typically, a derivative will include at least one inverted terminal repeat sequence (ITR), preferably more than one ITR, such as two ITRs or more. One or more of the ITRs may be derived from AAV genomes having different serotypes, or may be a chimeric or mutant ITR. A preferred mutant ITR is one having a deletion of a trs (terminal resolution site). This deletion allows for continued replication of the genome to generate a single-stranded genome which contains both coding and complementary sequences, i.e. a self-complementary AAV genome. This allows for bypass of DNA replication in the target cell, and so enables accelerated transgene expression.

The one or more ITRs will preferably flank the nucleotide sequence encoding the Complement Factor I or FHL1 at either end. The inclusion of one or more ITRs is preferred to aid concatamer formation of the vector of the invention in the nucleus of a host cell, for example following the conversion of single-stranded vector DNA into double-stranded DNA by the action of host cell DNA polymerases. The formation of such episomal concatamers

protects the vector construct during the life of the host cell, thereby allowing for prolonged expression of the transgene in vivo.

In preferred embodiments, ITR elements will be the only sequences retained from the native AAV genome in the derivative. Thus, a derivative will preferably not include the rep and/or cap genes of the native genome and any other sequences of the native genome. This is preferred for the reasons described above, and also to reduce the possibility of integration of the vector into the host cell genome. Additionally, reducing the size of the AAV genome allows for increased flexibility in incorporating other sequence elements (such as regulatory elements) within the vector in addition to the transgene.

The following portions could therefore be removed in a derivative of the invention: one inverted terminal repeat (ITR) sequence, the replication (rep) and capsid (cap) genes. However, in some embodiments, derivatives may additionally include one or more rep and/or cap genes or other viral sequences of an AAV genome. Naturally occurring AAV integrates with a high frequency at a specific site on human chromosome 19, and shows a negligible frequency of random integration, such that retention of an integrative capacity in the vector may be tolerated in a therapeutic setting.

Where a derivative comprises capsid proteins i.e. VP1, VP2 and/or VP3, the derivative may be a chimeric, shuffled or capsid-modified derivative of one or more naturally occurring AAVs. In particular, the invention encompasses the provision of capsid protein sequences from different serotypes, clades, clones, or isolates of AAV within the same vector (i.e. a pseudotyped vector).

Chimeric, shuffled or capsid-modified derivatives will be typically selected to provide one or more desired functionalities for the AAV vector. Thus, these derivatives may display increased efficiency of gene delivery, decreased immunogenicity (humoral or cellular), an altered tropism range and/or improved targeting of a particular cell type compared to an AAV vector comprising a naturally occurring AAV genome, such as that of AAV2. Increased efficiency of gene delivery may be effected by improved receptor or co-receptor binding at the cell surface, improved internalisation, improved trafficking within the cell and into the nucleus, improved uncoating of the viral particle and improved conversion of a single-stranded genome to double-stranded form. Increased efficiency may also relate to an altered tropism range or targeting of a specific cell population, such that the vector dose is not diluted by administration to tissues where it is not needed.

Chimeric capsid proteins include those generated by recombination between two or more capsid coding sequences of naturally occurring AAV serotypes. This may be performed for

example by a marker rescue approach in which non-infectious capsid sequences of one serotype are co-transfected with capsid sequences of a different serotype, and directed selection is used to select for capsid sequences having desired properties. The capsid sequences of the different serotypes can be altered by homologous recombination within the cell to produce novel chimeric capsid proteins.

Chimeric capsid proteins also include those generated by engineering of capsid protein sequences to transfer specific capsid protein domains, surface loops or specific amino acid residues between two or more capsid proteins, for example between two or more capsid proteins of different serotypes.

Shuffled or chimeric capsid proteins may also be generated by DNA shuffling or by error-prone PCR. Hybrid AAV capsid genes can be created by randomly fragmenting the sequences of related AAV genes e.g. those encoding capsid proteins of multiple different serotypes and then subsequently reassembling the fragments in a self-priming polymerase reaction, which may also cause crossovers in regions of sequence homology. A library of hybrid AAV genes created in this way by shuffling the capsid genes of several serotypes can be screened to identify viral clones having a desired functionality. Similarly, error prone PCR may be used to randomly mutate AAV capsid genes to create a diverse library of variants which may then be selected for a desired property.

The sequences of the capsid genes may also be genetically modified to introduce specific deletions, substitutions or insertions with respect to the native wild-type sequence. In particular, capsid genes may be modified by the insertion of a sequence of an unrelated protein or peptide within an open reading frame of a capsid coding sequence, or at the N- and/or C-terminus of a capsid coding sequence.

The unrelated protein or peptide may advantageously be one which acts as a ligand for a particular cell type, thereby conferring improved binding to a target cell or improving the specificity of targeting of the vector to a particular cell population. An example might include the use of RGD peptide to block uptake in the retinal pigment epithelium and thereby enhance transduction of surrounding retinal tissues (Cronin et al. (2008) ARVO Abstract: D1048). The unrelated protein may also be one which assists purification of the viral particle as part of the production process, i.e. an epitope or affinity tag. The site of insertion will typically be selected so as not to interfere with other functions of the viral particle e.g. internalisation, trafficking of the viral particle. The skilled person can identify suitable sites for insertion based on their common general knowledge. Particular sites are disclosed in Choi et al., referenced above.

The invention additionally encompasses the provision of sequences of an AAV genome in a different order and configuration to that of a native AAV genome. The invention also encompasses the replacement of one or more AAV sequences or genes with sequences from another virus or with chimeric genes composed of sequences from more than one virus. Such chimeric genes may be composed of sequences from two or more related viral proteins of different viral species.

The AAV vector of the invention may take the form of a nucleotide sequence comprising an AAV genome or derivative thereof and a sequence encoding the Complement Factor I or FHL1 transgene or derivatives thereof.

The AAV particles of the invention include transcapsidated forms wherein an AAV genome or derivative having an ITR of one serotype is packaged in the capsid of a different serotype. The AAV particles of the invention also include mosaic forms wherein a mixture of unmodified capsid proteins from two or more different serotypes makes up the viral capsid. The AAV particle also includes chemically modified forms bearing ligands adsorbed to the capsid surface. For example, such ligands may include antibodies for targeting a particular cell surface receptor.

Thus, for example, the AAV particles of the invention include those with an AAV2 genome and AAV2 capsid proteins (AAV2/2), those with an AAV2 genome and AAV5 capsid proteins (AAV2/5) and those with an AAV2 genome and AAV8 capsid proteins (AAV2/8), as well as those with an AAV2 genome and capsid proteins of more than one serotype.

The AAV vector may comprise multiple copies (e.g., 2, 3 etc.) of the nucleotide sequence referred to herein.

PROMOTERS AND REGULATORY SEQUENCES

The polynucleotide or vector of the invention may also include elements allowing for the expression of the Complement Factor I or FHL1 transgenes in vitro or in vivo. These may be referred to as expression control sequences. Thus, the polynucleotide or vector typically comprises expression control sequences (e.g. comprising a promoter sequence) operably linked to the nucleotide sequence encoding the transgene.

Any suitable promoter may be used, the selection of which may be readily made by the skilled person. The promoter sequence may be constitutively active (i.e. operational in any host cell background), or alternatively may be active only in a specific host cell environment, thus allowing for targeted expression of the transgene in a particular cell type (e.g. a tissue-

specific promoter). The promoter may show inducible expression in response to presence of another factor, for example a factor present in a host cell. In any event, where the vector is administered for therapy, it is preferred that the promoter should be functional in the target cell background.

In some embodiments, it is preferred that the promoter shows retinal-cell specific expression in order to allow for the transgene to only be expressed in retinal cell populations. Thus, expression from the promoter may be retinal-cell specific, for example confined only to cells of the neurosensory retina and retinal pigment epithelium.

Preferred promoters, which are not retinal-cell specific, include the chicken beta-actin (CBA) promoter, optionally in combination with a cytomegalovirus (CMV) enhancer element. An example promoter for use in the invention is a CAG promoter, for example the promoter used in the rAVE expression cassette (GeneDetect.com).

In preferred embodiments, the polynucleotide or vector comprises a CMV promoter.

An example CMV promoter sequence is:

GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGAC
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTT
ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGA
CTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGA
CTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTA
TATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAG
AAGACACCG

(SEQ ID NO: 13)

In some embodiments, the polynucleotide or vector comprises a promoter with a nucleotide sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 13. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the promoter represented by SEQ ID NO: 13.

In other embodiments, the polynucleotide or vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 13.

A further example promoter sequence is:

ATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGA
GTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGT
CAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTA
CATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTC
CCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGG
GGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGC

GGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAA
AAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTC
GCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC
GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCT
CCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGAC
GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCT
TCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATT

(SEQ ID NO: 5)

In some embodiments, the polynucleotide or vector comprises a promoter with a nucleotide sequence that has at least 75%, 80%, 85% 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 5. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the promoter represented by SEQ ID NO: 5.

In other embodiments, the polynucleotide or vector comprises a promoter with the nucleotide sequence of SEQ ID NO: 5.

Examples of promoters based on human sequences that would induce retina-specific gene expression include rhodopsin kinase for rods and cones (Allocca et al. (2007) J. Virol. 81: 11372-80), PR2.1 for cones only (Mancuso et al. (2009) Nature 461: 784-7) and/or RPE65 (Bainbridge et al. (2008) N. Engl. J. Med. 358: 2231-9) or VMD2 (Esumi et al. (2004) J. Biol. Chem. 279: 19064-73) for the retinal pigment epithelium.

The polynucleotide or vector of the invention may also comprise one or more additional regulatory sequences which may act pre- or post-transcriptionally. The regulatory sequence may be part of the native transgene locus or may be a heterologous regulatory sequence. The polynucleotide or vector of the invention may comprise portions of the 5'-UTR or 3'-UTR from the native transgene transcript.

Regulatory sequences are any sequences which facilitate expression of the transgene, i.e. act to increase expression of a transcript, improve nuclear export of mRNA or enhance its stability. Such regulatory sequences include for example enhancer elements, post-transcriptional regulatory elements and polyadenylation sites.

A preferred polyadenylation site is the Bovine Growth Hormone poly-A (bGH poly-A) signal.

An example Bovine Growth Hormone poly-A (bGH poly-A) signal is:

GTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACT
CCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGG
GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGC
TCTATGG

(SEQ ID NO: 14)

A further example Bovine Growth Hormone poly-A (bGH poly-A) signal is:

TCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTT
GACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAG
GTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
TGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGG

(SEQ ID NO: 6)

In some embodiments, the polynucleotide or vector comprises a polyadenylation signal with a nucleotide sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 14 or 6. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the polyadenylation signal represented by SEQ ID NO: 14 or 6.

In other embodiments, the polynucleotide or vector comprises a polyadenylation signal with the nucleotide sequence of SEQ ID NO: 14 or 6.

In the context of the polynucleotide or vector of the invention, such regulatory sequences will be cis-acting. However, the invention also encompasses the use of trans-acting regulatory sequences located on additional genetic constructs.

A preferred post-transcriptional regulatory element for use in a AAV vector of the invention is the woodchuck hepatitis post-transcriptional regulatory element (WPRE) or a variant thereof.

An example WPRE is:

ATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTAT
GTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGT
ATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTG
TGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTT
TCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGT
TGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCA
CCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCG
GCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGG
CCGCCTCCCCGC

(SEQ ID NO: 7)

WPRE is a tripartite element containing gamma, alpha and beta elements, in the given order. A shortened version of WPRE, which contains only minimal gamma and alpha elements (referred to as WPRE3; Choi, J.-H. et al. (2014) Molecular Brain 7: 17), may also be used in the invention.

An example WPRE3 sequence is:

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTA
TGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTG

TATAAATCCTGGTTAGTTCTTGCCACGGCGGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCT
CGGCTGTTGGGCACTGACAATTCCGTGGT

(SEQ ID NO: 15)

In some embodiments, the polynucleotide or vector comprises a post-transcriptional regulatory element with a nucleotide sequence that has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 15 or 7. Preferably, wherein the nucleotide sequence substantially retains the functional activity of the post-transcriptional regulatory element represented by SEQ ID NO: 15 or 7.

In other embodiments, the polynucleotide or vector comprises a post-transcriptional regulatory element with the nucleotide sequence of SEQ ID NO: 15 or 7.

Another regulatory sequence which may be used in a polynucleotide or vector of the invention is a scaffold-attachment region (SAR). Additional regulatory sequences may be readily selected by the skilled person.

METHOD OF ADMINISTRATION

The polynucleotide or vector of the invention may be administered systemically (for example by peripheral vein infusion) and may be administered locally (for example to the CNS system by intrathecal injection). In preferred embodiments, the polynucleotide or vector is administered intraocularly.

The term “intraocular” refers to the interior of the eye, thus intraocular administration relates to the administration to the interior of the eye of a subject

In some embodiments, the polynucleotide or vector is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection. In some embodiments, said administration is performed by a robot.

The volume of the medicament composition injected may, for example, be about 10-500 µL, for example about 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250 or 50-150 µL. The volume may, for example, be about 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 µL. Preferably, the volume of the medicament composition injected is 100 µL.

The skilled person will be familiar with and well able to carry out individual subretinal, direct retinal, suprachoroidal or intravitreal injections.

Preferably, the polynucleotide or vector is administered by subretinal injection.

In some embodiments, the polynucleotide, vector or pharmaceutical composition comprising the same is administered not more than once, or not more than twice, during the lifetime of a subject.

Subretinal injection

Subretinal injections are injections into the subretinal space, i.e. underneath the neurosensory retina. During a subretinal injection, the injected material is directed into, and creates a space between, the photoreceptor cell and retinal pigment epithelial (RPE) layers.

When the injection is carried out through a small retinotomy, a retinal detachment may be created. The detached, raised layer of the retina that is generated by the injected material is referred to as a "bleb".

The hole created by the subretinal injection must be sufficiently small that the injected solution does not significantly reflux back into the vitreous cavity after administration. Such reflux would be particularly problematic when a medicament is injected, because the effects of the medicament would be directed away from the target zone. Preferably, the injection creates a self-sealing entry point in the neurosensory retina, i.e. once the injection needle is removed, the hole created by the needle reseals such that very little or substantially no injected material is released through the hole.

To facilitate this process, specialist subretinal injection needles are commercially available (e.g. DORC 41G Teflon subretinal injection needle, Dutch Ophthalmic Research Center International BV, Zuidland, The Netherlands). These are needles designed to carry out subretinal injections.

Unless damage to the retina occurs during the injection, and as long as a sufficiently small needle is used, substantially all injected material remains localised between the detached neurosensory retina and the RPE at the site of the localised retinal detachment (i.e. does not reflux into the vitreous cavity). Indeed, the typical persistence of the bleb over a short time frame indicates that there is usually little escape of the injected material into the vitreous. The bleb may dissipate over a longer time frame as the injected material is absorbed.

Visualisations of the eye, in particular the retina, for example using optical coherence tomography, may be made pre-operatively.

The volume of the medicament composition injected may, for example, be about 10-500 µL, for example about 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250 or 50-150 µL. The volume may, for example, be about 10, 50, 100, 150, 200, 250, 300, 350,

400, 450 or 500 µL. Preferably, the volume of the medicament composition injected is 100 µL. Larger volumes may increase the risk of stretching the retina, while smaller volumes may be difficult to see.

Two-step subretinal injection

The polynucleotide or vector of the invention may be delivered with increased accuracy and safety by using a two-step method in which a localised retinal detachment is created by the subretinal injection of a first solution. The first solution does not comprise the polynucleotide or vector. A second subretinal injection is then used to deliver the medicament comprising the polynucleotide or vector into the subretinal fluid of the bleb created by the first subretinal injection. Because the injection delivering the medicament is not being used to detach the retina, a specific volume of solution may be injected in this second step.

In some embodiments, the subretinal injection of the vector comprises the steps:

(a) administering a solution to the subject by subretinal injection in an amount effective to at least partially detach the retina to form a subretinal bleb, wherein the solution does not comprise the polynucleotide or vector; and

(b) administering a medicament composition by subretinal injection into the bleb formed by step (a), wherein the medicament comprises the polynucleotide or vector.

The volume of solution injected in step (a) to at least partially detach the retina may be, for example, about 10-1000 µL, for example about 50-1000, 100-1000, 250-1000, 500-1000, 10-500, 50-500, 100-500, 250-500 µL. The volume may be, for example, about 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 µL.

The volume of the medicament composition injected in step (b) may be, for example, about 10-500 µL, for example about 50-500, 100-500, 200-500, 300-500, 400-500, 50-250, 100-250, 200-250 or 50-150 µL. The volume may be, for example, about 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 µL. Preferably, the volume of the medicament composition injected in step (b) is 100 µL. Larger volumes may increase the risk of stretching the retina, while smaller volumes may be difficult to see.

The solution that does not comprise the medicament (i.e. the “solution” of step (a)) may be similarly formulated to the solution that does comprise the medicament, as described below. A preferred solution that does not comprise the medicament is balanced saline solution (BSS) or a similar buffer solution matched to the pH and osmolality of the subretinal space.

Visualising the retina during surgery

Under certain circumstances, for example during end-stage retinal degenerations, identifying the retina is difficult because it is thin, transparent and difficult to see against the disrupted and heavily pigmented epithelium on which it sits. The use of a blue vital dye (e.g. Brilliant Peel®, Geuder; MembraneBlue-Dual®, Dorc) may facilitate the identification of the retinal hole made for the retinal detachment procedure (i.e. step (a) in the two-step subretinal injection method of the invention) so that the medicament can be administered through the same hole without the risk of reflux back into the vitreous cavity.

The use of the blue vital dye also identifies any regions of the retina where there is a thickened internal limiting membrane or epiretinal membrane, as injection through either of these structures would hinder clean access into the subretinal space. Furthermore, contraction of either of these structures in the immediate post-operative period could lead to stretching of the retinal entry hole, which could lead to reflux of the medicament into the vitreous cavity.

Suprachoroidal injection

The polynucleotide or vector of the invention may be delivered to the suprachoroidal space using an ab externo approach that utilises an microcatheter (see, for example, Peden et al. (2011) PLoS One 6(2): e17140). In this method a limbal conjunctival peritomy is performed to expose bare sclera, followed by sclerotomy to expose bare choroid. A microcatheter (such as the iTrack 250A from iScience Interventional, optionally connected to an illumination system such as the iLumin laser-diode based micro-illumination system (iScience Interventional)) is introduced into the suprachoroidal space and advanced posteriorly towards the optic disc. Following manipulation of the microcatheter tip into the desired position, injection of the polynucleotide or vector forms a bleb within the retina and choroid.

Thus, in some embodiments, the polynucleotide or vector is delivered suprachoroidally by a method comprising (i) introduction of a microcatheter into the suprachoroidal space; (ii) advancing the microcatheter within said space until the tip is in the proximity of the afflicted region of the retina; and (iii) injecting the polynucleotide or vector from the microcatheter tip to create a bleb.

In some embodiments, the above administration procedures are directly carried out by a robot.

PHARMACEUTICAL COMPOSITIONS AND INJECTED SOLUTIONS

The medicaments, for example polynucleotides or vectors, of the invention may be formulated into pharmaceutical compositions. These compositions may comprise, in addition to the medicament, a pharmaceutically acceptable carrier, diluent, excipient, buffer, stabiliser or other materials well known in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may be determined by the skilled person according to the route of administration, e.g. subretinal, direct retinal, suprachoroidal or intravitreal injection.

The pharmaceutical composition is typically in liquid form. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, magnesium chloride, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. In some cases, a surfactant, such as pluronic acid (PF68) 0.001% may be used.

For injection at the site of affliction, the active ingredient may be in the form of an aqueous solution which is pyrogen-free, and has suitable pH, isotonicity and stability. The skilled person is well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection or Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included as required.

For delayed release, the medicament may be included in a pharmaceutical composition which is formulated for slow release, such as in microcapsules formed from biocompatible polymers or in liposomal carrier systems according to methods known in the art.

METHOD OF TREATMENT

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment; although in the context of the invention references to preventing are more commonly associated with prophylactic treatment. Treatment may also include arresting progression in the severity of a disease.

The treatment of mammals, particularly humans, is preferred. However, both human and veterinary treatments are within the scope of the invention.

VARIANTS, DERIVATIVES, ANALOGUES, HOMOLOGUES AND FRAGMENTS

In addition to the specific proteins and nucleotides mentioned herein, the invention also encompasses the use of variants, derivatives, analogues, homologues and fragments thereof.

In the context of the invention, a variant of any given sequence is a sequence in which the specific sequence of residues (whether amino acid or nucleic acid residues) has been modified in such a manner that the polypeptide or polynucleotide in question substantially retains its function. A variant sequence can be obtained by addition, deletion, substitution, modification, replacement and/or variation of at least one residue present in the naturally-occurring protein.

The term “derivative” as used herein, in relation to proteins or polypeptides of the invention includes any substitution of, variation of, modification of, replacement of, deletion of and/or addition of one (or more) amino acid residues from or to the sequence providing that the resultant protein or polypeptide substantially retains at least one of its endogenous functions.

The term “analogue” as used herein, in relation to polypeptides or polynucleotides includes any mimetic, that is, a chemical compound that possesses at least one of the endogenous functions of the polypeptides or polynucleotides which it mimics.

Typically, amino acid substitutions may be made, for example from 1, 2 or 3 to 10 or 20 substitutions provided that the modified sequence substantially retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

Proteins used in the invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues as long as the endogenous function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include asparagine, glutamine, serine, threonine and tyrosine.

Conservative substitutions may be made, for example according to the table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R H
AROMATIC		F W Y

The term “homologue” as used herein means an entity having a certain homology with the wild type amino acid sequence and the wild type nucleotide sequence. The term “homology” can be equated with “identity”.

A homologous sequence may include an amino acid sequence which may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% identical, preferably at least 95% or 97% or 99% identical to the subject sequence. Typically, the homologues will comprise the same active sites etc. as the subject amino acid sequence. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the invention it is preferred to express homology in terms of sequence identity.

A homologous sequence may include a nucleotide sequence which may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90% identical, preferably at least 95% or 97% or 99% identical to the subject sequence. Although homology can also be considered in terms of similarity, in the context of the invention it is preferred to express homology in terms of sequence identity.

Preferably, reference to a sequence which has a percent identity to any one of the SEQ ID NOs detailed herein refers to a sequence which has the stated percent identity over the entire length of the SEQ ID NO referred to.

Homology comparisons can be conducted by eye or, more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate percentage homology or identity between two or more sequences.

Percentage homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an “ungapped” alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion in the nucleotide sequence may cause the following codons to be put out of alignment, thus potentially resulting in a large reduction in percent homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without

penalising unduly the overall homology score. This is achieved by inserting “gaps” in the sequence alignment to try to maximise local homology.

However, these more complex methods assign “gap penalties” to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. “Affine gap costs” are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum percentage homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux et al. (1984) Nucleic Acids Res. 12: 387). Examples of other software that can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al. (1999) ibid – Ch. 18), FASTA (Atschul et al. (1990) J. Mol. Biol. 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al. (1999) ibid, pages 7-58 to 7-60). However, for some applications, it is preferred to use the GCG Bestfit program. Another tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequences (see FEMS Microbiol. Lett. (1999) 174: 247-50; FEMS Microbiol. Lett. (1999) 177: 187-8).

Although the final percent homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix – the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see the user manual for further details). For some applications, it is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate percent homology, preferably percent sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

"Fragments" of full length Complement Factor I or FHL1 are also variants and the term typically refers to a selected region of the polypeptide or polynucleotide that is of interest either functionally or, for example, in an assay. "Fragment" thus refers to an amino acid or nucleic acid sequence that is a portion of a full-length polypeptide or polynucleotide.

Such variants may be prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site may be made. The flanking regions will contain convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the synthetic DNA ligated into the cut. The DNA is then expressed in accordance with the invention to make the encoded protein. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used.

The skilled person will understand that they can combine all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

Preferred features and embodiments of the invention will now be described by way of non-limiting examples.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press; Ausubel, F.M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, Ch. 9, 13 and 16, John Wiley & Sons; Roe, B., Crabtree, J. and Kahn, A. (1996) DNA Isolation and Sequencing: Essential Techniques, John Wiley & Sons; Polak, J.M. and McGee, J.O'D. (1990) In Situ Hybridization: Principles and Practice, Oxford University Press; Gait, M.J. (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; and Lilley, D.M. and Dahlberg, J.E. (1992) Methods in Enzymology: DNA Structures Part A: Synthesis

and Physical Analysis of DNA, Academic Press. Each of these general texts is herein incorporated by reference.

EXAMPLES

EXAMPLE 1

Codon optimisation

Nucleotide sequences encoding Complement Factor I (CFI) and Complement Factor H-like Protein 1 (FHL1) were codon optimised using a range of approaches summarised in Table 1.

Table 1

RC number	Transgene	Codon Optimisation Tool	Basic or manually edited	Size of AAV
RC128	CFI	GeneArt (ThermoFisher)	Basic	3948bp
RC129	CFI	GeneArt (ThermoFisher)	Manually edited	3948bp
RC130	CFI	GenScript	Basic	3948bp
RC131	CFI	GenScript	Manually edited	3948bp
RC132	CFI	IDT	Basic	3948bp
RC133	CFI	IDT	Manually edited	3948bp
RC134	CFI	JCat	Basic	3948bp
RC135	CFI	JCat	Manually edited	3948bp
RC136	CFI	COOL	Basic	3948bp
RC137	CFI	COOL	Manually edited	3948bp
RC138	FHL-1	GeneArt (ThermoFisher)	Basic	3546bp
RC139	FHL-1	GeneArt (ThermoFisher)	Manually edited	3546bp
RC140	FHL-1	GenScript	Basic	3546bp
RC141	FHL-1	GenScript	Manually edited	3546bp
RC142	FHL-1	IDT	Basic	3546bp
RC143	FHL-1	IDT	Manually edited	3546bp
RC144	FHL-1	JCat	Basic	3546bp
RC145	FHL-1	JCat	Manually edited	3546bp
RC146	FHL-1	COOL	Basic	3546bp
RC147	FHL-1	COOL	Manually edited	3546bp

For the “basic” codon optimisation, the sequence of CFI or FHL-1 was entered into 5 online codon optimisation tools:

1. GeneArt (https://www.thermofisher.com/uk/en/home/life-science/cloning/gene-synthesis/geneart-gene-synthesis/geneoptimizer.html)
2. GenScript (https://www.genscript.com/quick_order/gene_services_gene_synthesis)
3. IDT (https://eu.idtdna.com/CodonOpt)
4. JCat (http://www.jcat.de/)
5. COOL (http://cool.syncti.org/setup_input_sequence_create_wf1.php?=Start+Using+Codon+Optimization+On-Line+%3E%3E%3E)

The standard human genetic code was used for all tools.

For tools 1-4 above, one sequence was generated from each tool.

For tool 5, default settings were used and the target expression host was set to *Homo sapiens*. In addition, 39 genes that are highly expressed in the RPE (Table 2) were input into the tool (based on Table 4 of Booij, J.C. et al. (2010) PLoS One 5: e9341).

Table 2

Gene symbol	Genbank ID	HUGO
C6orf105	NM_032744	ADTRP
BEST1	NM_004183	BEST1
TMEM27	NM_020665	TMEM27
LRP8	NM_004631	LRP8
LGI1	NM_005097	LGI1
FAM40B	AB032996	STRIP2
ERMN	AB033015	ERMN
LRAT	NM_004744	LRAT
RLBP1	NM_000326	RLBP1
DUSP6	NM_001946	DUSP6
RBP1	NM_002899	RBP1
SLC16A3	NM_004207	SLC16A3
WFDC1	NM_021197	WFDC1
KIAA0953	AF131834	EFR3B
CA14	NM_012113	CA14
RGR	NM_002921	RGR
STRA6	NM_022369	STRA6
RDH5	NM_002905	RDH5
BMP4	NM_001202	BMP4
CXCL14	NM_004887	CXCL14
LHX2	NM_004789	LHX2
C1QTNF5	NM_015645	C1QTNF5
SLC6A20	NM_020208	SLC6A20
SLC16A8	NM_013356	SLC16A8
CDH3	NM_001793	CDH3
FRZB	NM_001463	FRZB
SERPINF1	NM_002615	SERPINF1
SPOCK1	NM_004598	SPOCK1
LMO1	NM_002315	LMO1
RDH11	NM_016026	RDH11
SFRP5	NM_003015	SFRP5
SGK1	NM_005627	SGK1
KRT18	NM_000224	KRT18
EZR	NM_003379	EZR

DHCR7	NM_001360	DHCR7
ITGAV	NM_002210	ITGAV
GALNT11	NM_022087	GALNT11
PCP4	NM_006198	PCP4
BASP1	NM_006317	BASP1

Tool 5 generated 70 optimised sequences for CFI and 55 for FHL-1, the top ranking sequence was used.

For the "manual" codon optimisation, the five basic CFI and FHL-1 codon optimised sequences generated above were subjected to manual optimisation to eliminate cryptic splice sites, microRNA binding sites, to remove tandem duplicate codons and to check GC content.

Cryptic Splice Site Removal

Cryptic splice sites were identified using the www.Fruitfly.org tool. A cut-off value of 0.4 was used for analysis, but only sequences scoring >0.75 were modified.

Splice sites were removed by changing the GT of the donor site or the AG of the acceptor site wherever possible. When not possible (e.g. for sequences encoding valine), the 5' adjacent base was changed.

All modified sequences were then analysed with www.Fruitfly.org tool to confirm that all splice sites had either been removed or reduced to below the 0.75 threshold.

MicroRNA binding site removal

MicroRNAs were identified using the www.Genecards.org tool.

For CFI, the following miRNA binding sites were identified: hsa-mir-335-5p, hsa-mir-181a-5p, hsa-mir-26b-5p.

For FHL-1, the following miRNA binding sites were identified (based on the sequence of Complement Factor H, CFH): hsa-mir-146a-5p.

Each codon optimised sequence (after splice site removal if necessary) was then passed through the STarMir tool (http://sfold.wadsworth.org/cgi-bin/starmirtest2.pl) to see if the miRNA sites were still present. Any miRNA sites the were identified with a logistical probability of >0.75 were modified.

Tandem duplicate codon removal

All sequences were manually checked for tandem duplicate codons. Where these were found, the second codon was changed to the next most commonly used codon in *Homo sapiens* (using the SnapGene codon usage table).

The wild type and codon optimised sequences are detailed below:

GT005: CFI wild type sequence:

ATGAAGCTTCTTCATGTTTTCCTGTTATTTCTGTGCTTCCACTTAAGGTTTTGCAAGGTCACTTATACATCT
CAAGAGGATCTGGTGGAGAAAAAGTGCTTAGCAAAAAAATATACTCACCTCTCCTGCGATAAAGTCTTCTGC
CAGCCATGGCAGAGATGCATTGAGGGCACCTGTGTTTGTAAACTACCGTATCAGTGCCCAAAGAATGGCACT
GCAGTGTGTGCAACTAACAGGAGAAGCTTCCCAACATACTGTCAACAAAAGAGTTTGGAATGTCTTCATCCA
GGGACAAAGTTTTTAAATAACGGAACATGCACAGCCGAAGGAAAGTTTAGTGTTTCCTTGAAGCATGGAAAT
ACAGATTCAGAGGGAATAGTTGAAGTAAAACTTGTGGACCAAGATAAGACAATGTTCATATGCAAAAGCAGC
TGGAGCATGAGGGAAGCCAACGTGGCCTGCCTTGACCTTGGGTTTCAACAAGGTGCTGATACTCAAAGAAGG
TTTAAGTTGTCTGATCTCTCTATAAATTCCACTGAATGTCTACATGTGCATTGCCGAGGATTAGAGACCAGT
TTGGCTGAATGTACTTTTACTAAGAGAAGAACTATGGGTTACCAGGATTTCGCTGATGTGGTTTGTTATACA
CAGAAAGCAGATTCTCCAATGGATGACTTCTTTCAGTGTGTGAATGGGAAATACATTTCTCAGATGAAAGCC
TGTGATGGTATCAATGATTGTGGAGACCAAAGTGATGAACTGTGTTGTAAAGCATGCCAAGGCAAAGGCTTC
CATTGCAAATCGGGTGTTTGCATTCCAAGCCAGTATCAATGCAATGGTGAGGTGGACTGCATTACAGGGGAA
GATGAAGTTGGCTGTGCAGGCTTTGCATCTGTGGCTCAAGAAGAAACAGAAATTTTGACTGCTGACATGGAT
GCAGAAAGAAGACGGATAAAATCATTATTACCTAAACTATCTTGTGGAGTTAAAAACAGAATGCACATTCGA
AGGAAACGAATTGTGGGAGGAAAGCGAGCACAACTGGGAGACCTCCCATGGCAGGTGGCAATTAAGGATGCC
AGTGGAATCACCTGTGGGGGAATTTATATTGGTGGCTGTTGGATTCTGACTGCTGCACATTGTCTCAGAGCC
AGTAAAACTCATCGTTACCAAATATGGACAACAGTAGTAGACTGGATACACCCCGACCTTAAACGTATAGTA
ATTGAATACGTGGATAGAATTATTTTCCATGAAAACTACAATGCAGGCACTTACCAAAATGACATCGCTTTG
ATTGAAATGAAAAAAGACGGAAACAAAAAAGATTGTGAGCTGCCTCGTTCCATCCCTGCCTGTGTCCCCTGG
GTCTTTTCACTTCAGTGGGGTGAAGTTAAACTAATAAGCAACTGCTCTAAGTTTTACGGAAATCGTTTCTAT
GAAAAAGAAATGGAATGTGCAGGTACATATGATGGTTCCATCGATGCCTGTAAAGGGGACTCTGGAGGCCCC
TTAGTCTGTATGGATGCCAACAATGTGACTTATGTCTGGGGTGTTGTGAGTTGGGGGGAAAACTGTGGAAAA
CCAGAGTTCCCAGGTGTTTACACCAAAGTGGCCAATTATTTTGACTGGATTAGCTACCATGTAGGAAGGCCT
TTTATTTCTCAGTACAATGTATAA

(SEQ ID NO: 16)

RC001: FHL-1 wild type sequence:

ATGAGACTTCTAGCAAAGATTATTTGCCTTATGTTATGGGCTATTTGTGTAGCAGAAGATTGCAATGAACTT
CCTCCAAGAAGAAATACAGAAATTCTGACAGGTTCCTGGTCTGACCAAACATATCCAGAAGGCACCCAGGCT
ATCTATAAATGCCGCCCTGGATATAGATCTCTTGGAAATATAATAATGGTATGCAGGAAGGGAGAATGGGTT
GCTCTTAATCCATTAAGGAAATGTCAGAAAAGGCCCTGTGGACATCCTGGAGATACTCCTTTTGGTACTTTT
ACCCTTACAGGAGGAAATGTGTTTGAATATGGTGTAAAAGCTGTGTATACATGTAATGAGGGGTATCAATTG
CTAGGTGAGATTAATTACCGTGAATGTGACACAGATGGATGGACCAATGATATTCCTATATGTGAAGTTGTG
AAGTGTTTACCAGTGACAGCACCAGAGAATGGAAAAATTGTCAGTAGTGCAATGGAACCAGATCGGGAATAC
CATTTTGGACAAGCAGTACGGTTTGTATGTAACTCAGGCTACAAGATTGAAGGAGATGAAGAAATGCATTGT
TCAGACGATGGTTTTTGGAGTAAAGAGAAACCAAAGTGTGTGGAAATTTCATGCAAATCCCCAGATGTTATA
AATGGATCTCCTATATCTCAGAAGATTATTTATAAGGAGAATGAACGATTTCAATATAAATGTAACATGGGT
TATGAATACAGTGAAAGAGGAGATGCTGTATGCACTGAATCTGGATGGCGTCCGTTGCCTTCATGTGAAGAA
AAATCATGTGATAATCCTTATATTCCAAATGGTGACTACTCACCTTTAAGGATTAAACACAGAACTGGAGAT
GAAATCACGTACCAGTGTAGAAATGGTTTTTATCCTGCAACCCGGGGAAATACAGCaAAATGCACAAGTACT
GGCTGGATACCTGCTCCGAGATGTACCTTGAAACCTTGTGATTATCCAGACATTAAACATGGAGGTCTATAT
CATGAGAATATGCGTAGACCATACTTTCCAGTAGCTGTAGGAAAATATTACTCCTATTACTGTGATGAACAT
TTTGAGACTCCGTCAGGAAGTTACTGGGATCACATTCATTGCACACAAGATGGATGGTCGCCAGCAGTACCA
TGCCTCAGAAAATGTTATTTTCCTTATTTGGAAAATGGATATAATCAAAATTATGGAAGAAAGTTTGTACAG
GGTAAATCTATAGACGTTGCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAGTTACATGTATG
GAGAATGGCTGGTCTCCTACTCCCAGATGCATCCGTGTCAGCTTTACCCTCTGA

(SEQ ID NO: 17)

RC128: CFI GeneArt – Basic:

ATGAAGCTGCTGCATGTGTTTCTGCTGTTCCTCTGCTTCCACCTGAGGTTCTGCAAAGTGACCTACACCAGC
CAAGAGGACCTGGTGGAAAAGAAGTGCCTGGCCAAGAAGTACACCCACCTGAGCTGCGACAAGGTGTTCTGC
CAGCCTTGGCAGAGATGCATCGAGGGCACCTGTGTGTGCAAGCTGCCCTATCAGTGCCCCAAGAATGGCACA
GCCGTGTGCGCCACCAACAGAAGAAGCTTCCCTACCTACTGCCAGCAGAAAAGCCTGGAATGTCTGCACCCC
GGCACCAAGTTTCTGAACAACGGCACCTGTACCGCCGAGGGCAAGTTTAGCGTGTCCCTGAAGCACGGCAAC
ACCGACTCTGAGGGCATCGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATGTTCATCTGCAAGAGCAGC
TGGTCCATGCGCGAGGCCAATGTGGCTTGTCTGGATCTGGGATTCCAGCAGGGCGCCGACACACAGAGAAGA
TTCAAGCTGAGCGACCTGAGCATCAACAGCACCGAGTGCCTGCATGTGCACTGTAGAGGCCTGGAAACAAGC
CTGGCCGAGTGCACCTTCACCAAGAGAAGGACCATGGGCTACCAGGACTTCGCCGACGTCGTGTGCTACACC
CAGAAAGCCGACTCTCCCATGGACGATTTCTTCCAGTGCGTGAACGGCAAGTACATCAGCCAGATGAAGGCC
TGCGACGGCATCAACGATTGCGGCGATCAGAGCGACGAGCTGTGCTGCAAAGCCTGTCAAGGCAAGGGCTTC
CACTGCAAGTCCGGCGTGTGTATCCCTAGCCAGTACCAGTGCAATGGCGAGGTGGACTGTATCACCGGCGAG
GATGAAGTGGGCTGTGCCGGATTTGCCAGCGTGGCCCAAGAGGAAACCGAGATCCTGACCGCCGATATGGAC
GCCGAGCGGCGGAGAATCAAAAGCCTGCTGCCTAAGCTGTCCTGCGGCGTGAAGAACCGGATGCACATCCGG
CGCAAGAGAATCGTCGGAGGCAAAAGAGCACAGCTGGGCGATCTGCCTTGGCAAGTGGCCATCAAGGATGCC
AGCGGCATCACATGTGGCGGCATCTACATCGGCGGCTGCTGGATTCTGACAGCCGCTCATTGTCTGCGGGCC
AGCAAGACCCACCGGTATCAGATCTGGACCACCGTGGTGGACTGGATTCACCCCGACCTGAAGCGGATCGTG
ATCGAGTACGTGGACCGGATCATCTTCCACGAGAACTACAACGCCGGCACCTACCAGAACGATATCGCCCTG
ATCGAGATGAAGAAGGACGGGAACAAGAAGGACTGCGAGCTGCCTAGATCTATCCCCGCCTGTGTTCCTTGG
AGCCCCTACCTGTTCCAGCCTAACGATACCTGCATCGTGTCCGGCTGGGGCAGAGAGAAGGATAACGAGAGG
GTGTTCAGCCTGCAGTGGGGCGAAGTGAAACTGATCAGCAACTGCAGCAAGTTCTACGGCAACCGGTTCTAC
GAGAAAGAAATGGAATGCGCCGGCACATACGACGGCTCCATCGATGCCTGTAAAGGCGATTCTGGCGGCCCT
CTCGTGTGCATGGATGCCAACAATGTGACCTACGTGTGGGGCGTCGTGTCCTGGGGAGAGAATTGTGGCAAG
CCTGAGTTCCCCGGCGTGTACACCAAGGTGGCCAACTACTTCGACTGGATCAGCTACCACGTGGGCAGACCC
TTTATCAGCCAGTACAACGTGTGA

(SEQ ID NO: 18)

RC129: CFI GeneArt – Manually optimised:

ATGAAGCTGCTCCATGTGTTTCTGCTCTTCCTCTGCTTCCACCTGAGGTTCTGCAAAGTGACCTACACCAGC
CAAGAGGACCTGGTGGAAAAGAAATGCCTGGCCAAGAAATACACCCACCTGAGCTGCGACAAGGTGTTCTGC
CAGCCTTGGCAGAGATGCATCGAGGGCACCTGTGTGTGCAAGCTGCCCTATCAGTGCCCCAAGAATGGCACA
GCCGTGTGCGCTACAAACAGAAGGAGCTTCCCTACCTACTGCCAGCAAAAAAGCCTGGAGTGCCTGCACCCC
GGCACCAAGTTTCTGAACAATGGCACCTGTACCGCCGAGGGCAAGTTTAGCGTGTCCCTGAAGCACGGCAAC
ACCGACTCTGAGGGCATCGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATGTTCATCTGCAAGAGCTCC
TGGTCCATGCGCGAGGCCAATGTGGCTTGTCTGGATCTGGGATTCCAGCAAGGCGCCGACACACAGAGAAGG
TTCAAGCTGAGCGACCTGAGCATCAACAGCACCGAGTGCCTGCATGTGCACTGTAGAGGCCTGGAAACAAGC
CTGGCCGAGTGCACCTTCACCAAGAGAAGGACCATGGGCTACCAGGACTTCGCCGACGTCGTGTGCTACACC
CAGAAAGCCGACTCTCCCATGGACGATTTCTTTCAGTGCGTGAACGGCAAGTACATCAGCCAGATGAAGGCC
TGCGACGGCATCAACGATTGCGGCGATCAGAGCGACGAGCTGTGCTGTAAAGCCTGTCAAGGCAAGGGCTTC
CACTGCAAGTCCGGCGTGTGTATCCCTAGCCAGTACCAGTGCAATGGCGAGGTGGACTGTATCACCGGCGAG
GATGAAGTGGGCTGTGCCGGATTTGCCAGCGTGGCCCAAGAGGAAACCGAGATCCTGACCGCCGATATGGAC
GCCGAGCGGAGGAGAATCAAAAGCCTGCTCCCTAAGCTGTCCTGCGGCGTGAAGAACCGGATGCACATCCGG
CGCAAGAGAATCGTCGGAGGCAAAAGAGCACAGCTGGGCGATCTGCCTTGGCAAGTGGCCATCAAGGATGCC
AGCGGCATCACATGTGGCGGGATCTACATCGGCGGATGCTGGATTCTGACAGCCGCTCATTGTCTGCGGGCC
AGCAAGACCCACCGGTATCAGATCTGGACCACAGTGGTCGACTGGATTCACCCCGACCTGAAGCGGATCGTG
ATCGAGTACGTGGACCGGATCATTTTCCACGAGAACTACAACGCCGGCACCTACCAGAACGATATCGCCCTG
ATCGAGATGAAAAAGGACGGGAACAAGAAAGACTGCGAGCTGCCTAGATCTATCCCCGCCTGTGTTCCTTGG
AGCCCCTACCTGTTCCAGCCTAACGATACCTGCATCGTGTCCGGCTGGGGCAGAGAGAAGGATAACGAGAGG
GTGTTCAGCCTGCAGTGGGGCGAAGTGAAACTGATCAGCAACTGCAGCAAGTTCTACGGCAACCGGTTCTAC
GAGAAAGAAATGGAATGCGCCGGCACATACGACGGCTCCATCGATGCCTGTAAAGGCGATTCTGGCGGACCT
CTCGTGTGCATGGATGCCAACAATGTGACCTACGTGTGGGGCGTCGTGTCCTGGGGAGAGAATTGTGGCAAG
CCTGAGTTCCCCGGCGTGTACACCAAGGTGGCCAACTACTTCGACTGGATCAGCTACCACGTGGGCAGACCC
TTTATCAGCCAGTACAACGTGTGA

(SEQ ID NO: 19)

RC130: CFI Genscript – Basic:

ATGAAGCTGCTGCATGTCTTTCTGCTGTTTCTGTGCTTCCATCTGAGGTTCTGCAAGGTCACTTACACTAGC
CAGGAGGATCTGGTCGAGAAGAAGTGTCTGGCCAAGAAGTACACACACCTGAGCTGCGACAAGGTGTTCTGT
CAGCCTTGGCAGCGGTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCTTACCAGTGCCCAAAGAACGGCACC
GCCGTGTGCGCCACAAATCGGAGATCTTTTCCAACATATTGCCAGCAGAAGAGCCTGGAGTGTCTGCACCCC
GGCACCAAGTTCCTGAACAATGGCACCTGCACAGCCGAGGGCAAGTTTTCTGTGAGCCTGAAGCACGGCAAC
ACAGATAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGATAAGACCATGTTCATCTGTAAGAGCAGC
TGGTCCATGAGGGAGGCAAACGTGGCATGCCTGGATCTGGGATTCCAGCAGGGAGCAGACACACAGAGGCGC
TTTAAGCTGTCCGACCTGTCTATCAATAGCACCGAGTGCCTGCACGTGCACTGTAGGGGCCTGGAGACATCC
CTGGCAGAGTGCACCTTCACAAAGCGGAGAACAATGGGCTACCAGGACTTTGCCGACGTGGTGTGCTATACC
CAGAAGGCCGATAGCCCTATGGACGATTTCTTTCAGTGCGTGAACGGCAAGTATATCTCCCAGATGAAGGCC
TGCGACGGCATCAATGACTGTGGCGATCAGTCTGACGAGCTGTGCTGTAAGGCCTGTCAGGGCAAGGGCTTC
CACTGCAAGAGCGGCGTGTGCATCCCTTCCCAGTACCAGTGCAACGGCGAGGTGGATTGTATCACAGGAGAG
GACGAAGTGGGATGCGCAGGATTTGCATCTGTGGCACAGGAGGAGACAGAGATCCTGACAGCCGACATGGAT
GCCGAGAGGCGCCGGATCAAGTCTCTGCTGCCTAAGCTGAGCTGTGGCGTGAAGAATCGGATGCACATCAGA
AGGAAGCGCATCGTGGGAGGCAAGCGGGCCCAGCTGGGCGATCTGCCCTGGCAGGTGGCCATCAAGGACGCC
TCTGGCATCACCTGCGGCGGCATCTACATCGGCGGCTGTTGGATTCTGACCGCAGCACACTGCCTGAGAGCA
AGCAAGACACACAGGTATCAGATCTGGACCACAGTGGTGGATTGGATTCACCCAGACCTGAAGAGAATCGTG
ATCGAGTACGTGGATAGGATCATCTTCCACGAGAACTACAATGCCGGCACATATCAGAACGACATCGCCCTG
ATCGAGATGAAGAAGGATGGCAATAAGAAGGACTGTGAGCTGCCCAGATCCATCCCTGCATGCGTGCCCTGG
AGCCCCTATCTGTTCCAGCCCAACGATACCTGCATCGTGTCCGGATGGGGAAGGGAGAAGGACAATGAGCGG
GTGTTTTCTCTGCAGTGGGGCGAGGTGAAGCTGATCTCCAACTGTTCTAAGTTCTACGGCAATAGGTTTTAT
GAGAAGGAGATGGAGTGCGCCGGCACCTACGATGGCAGCATCGACGCCTGTAAGGGCGATTCCGGAGGACCA
CTGGTGTGCATGGACGCAAACAATGTGACATACGTGTGGGGAGTGGTGTCCTGGGGAGAGAACTGCGGCAAG
CCAGAGTTTCCCGGCGTGTATACCAAGGTGGCCAATTATTTTGATTGGATTTCATACCATGTCGGGAGACCA
TTCATTAGTCAGTACAACGTGTGA

(SEQ ID NO: 20)

RC131: CFI Genscript – Manually optimised:

ATGAAGCTGCTCCATGTCTTTCTGCTCTTTCTGTGCTTCCATCTGAGGTTCTGCAAGGTCACTTACACTAGC
CAGGAGGATCTGGTCGAGAAGAAATGTCTGGCCAAGAAATACACACACCTGAGCTGCGACAAGGTGTTCTGT
CAGCCTTGGCAGCGGTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCTTACCAGTGCCCAAAGAACGGCACC
GCCGTGTGCGCCACAAATCGGAGATCTTTTCCAACATATTGCCAGCAAAAGAGCCTGGAGTGTCTGCACCCC
GGCACCAAGTTCCTGAACAATGGCACCTGCACAGCCGAGGGCAAGTTTTCTGTGAGCCTGAAGCACGGCAAC
ACAGATAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGATAAGACCATGTTCATCTGTAAGAGCTCC
TGGTCCATGAGGGAGGCAAACGTGGCATGCCTGGATCTGGGATTCCAGCAAGGAGCAGACACACAGAGGCGC
TTTAAGCTGTCCGATCTGAGTATCAATAGCACCGAGTGCCTGCACGTGCACTGTAGGGGCCTGGAGACATCC
CTGGCAGAGTGCACCTTCACAAAGCGGAGAACAATGGGCTACCAGGACTTTGCCGACGTGGTCTGCTATACC
CAGAAGGCCGATAGCCCTATGGACGATTTCTTTCAGTGCGTGAACGGCAAGTATATCTCCCAGATGAAGGCC
TGCGACGGCATCAATGACTGTGGCGATCAGTCTGACGAGCTGTGCTGTAAGGCCTGTCAGGGCAAGGGCTTC
CACTGCAAGAGCGGCGTGTGCATCCCTTCCCAGTACCAGTGCAACGGCGAGGTGGATTGTATCACAGGAGAG
GACGAAGTGGGATGCGCAGGATTTGCATCTGTGGCACAGGAGGAAACAGAGATCCTGACAGCCGACATGGAT
GCCGAGAGGCGCCGGATCAAGTCTCTGCTCCCTAAGCTGAGCTGTGGCGTGAAGAATCGGATGCACATCAGA
AGGAAGCGCATCGTGGGAGGCAAGCGGGCCCAGCTGGGCGATCTGCCCTGGCAGGTGGCCATCAAGGACGCC
TCTGGCATCACCTGCGGCGGGATCTACATCGGCGGATGTTGGATTCTGACCGCAGCCCACTGCCTGAGAGCA
AGCAAGACACACAGATATCAGATCTGGACCACAGTGGTCGATTGGATTCACCCAGACCTGAAGAGAATCGTG
ATCGAGTACGTGGATAGGATCATTTTCCACGAGAATTACAATGCTGGCACATATCAGAATGATATCGCTCTC
ATCGAGATGAAGAAAGATGGCAATAAGAAAGACTGTGAGCTGCCCAGATCCATCCCTGCATGCGTGCCCTGG
AGCCCCTATCTGTTCCAGCCCAACGATACCTGCATCGTGTCCGGATGGGGAAGGGAGAAGGACAATGAGCGG
GTGTTTTCTCTGCAGTGGGGCGAGGTGAAGCTGATCTCCAACTGTTCTAAGTTCTACGGCAATAGGTTTTAT
GAGAAGGAGATGGAGTGCGCCGGCACCTACGATGGCAGCATCGACGCCTGTAAGGGCGATTCCGGAGGCCCA
CTGGTGTGCATGGACGCAAACAATGTGACATACGTGTGGGGAGTGGTCTCCTGGGGAGAGAACTGCGGCAAG
CCAGAGTTTCCCGGCGTGTATACCAAGGTGGCCAATTATTTTGATTGGATTTCATACCATGTCGGGAGACCA
TTCATTAGTCAATACAACGTTTGA

(SEQ ID NO: 21)

RC132: CFI IDT – Basic:

ATGAAGCTCCTCCACGTCTTCTTGTTGTTTCTCTGTTTCCACCTGAGATTTTGCAAAGTAACTTACACCAGT
CAAGAAGACTTGGTCGAGAAGAAGTGTCTCGCCAAAAAGTATACTCACCTGAGCTGTGATAAAGTGTTCTGT
CAGCCGTGGCAGCGCTGCATTGAGGGTACATGTGTCTGTAAACTGCCTTATCAGTGTCCGAAGAACGGTACG
GCTGTCTGTGCTACTAACAGACGGTCTTTTCCTACTTATTGCCAGCAGAAGAGTTTGGAATGTCTCCACCCT
GGTACCAAGTTTCTCAACAATGGCACCTGTACTGCTGAAGGTAAATTCTCCGTCAGTCTCAAGCATGGTAAC
ACTGACAGTGAAGGGATAGTAGAGGTAAAGTTGGTTGACCAGGACAAGACGATGTTCATATGCAAGTCAAGC
TGGTCCATGCGCGAGGCGAATGTCGCTTGTCTTGATTTGGGCTTCCAGCAAGGGGCAGACACACAGAGAAGA
TTCAAATTGAGCGACCTGAGTATAAATTCAACCGAGTGCCTCCATGTACATTGCAGAGGGCTCGAGACTTCA
CTTGCCGAATGTACATTTACGAAGAGGCGGACTATGGGATATCAGGACTTTGCCGACGTAGTATGTTATACT
CAGAAAGCAGACAGTCCTATGGATGACTTTTTCCAATGCGTCAACGGCAAATACATCAGTCAAATGAAAGCG
TGCGACGGTATCAACGATTGTGGTGACCAGTCTGATGAGCTTTGCTGTAAAGCATGTCAAGGAAAGGGGTTC
CATTGCAAGAGTGGTGTATGTATTCCCTCACAATATCAGTGCAATGGGGAAGTCGATTGCATAACAGGTGAG
GATGAGGTGGGCTGCGCGGGATTTGCTTCTGTGGCGCAAGAGGAGACTGAGATCCTTACAGCGGATATGGAC
GCCGAACGAAGACGCATCAAATCTCTCCTTCCCAAACTTTCATGCGGCGTCAAAAACCGAATGCATATACGC
AGGAAGAGAATTGTTGGGGGAAAGCGGGCACAGCTGGGCGACCTCCCCTGGCAAGTTGCAATAAAGGATGCA
AGTGGGATAACGTGCGGGGGCATCTACATCGGGGGGTGCTGGATCTTGACTGCCGCCCACTGTCTTAGAGCC
TCTAAGACCCATAGGTACCAAATCTGGACAACTGTAGTTGACTGGATACATCCGGACCTTAAACGCATAGTT
ATTGAATACGTTGACCGCATAATATTTCATGAGAATTATAACGCGGGTACCTATCAGAATGACATCGCCCTC
ATCGAGATGAAAAAAGACGGGAATAAAAAGGACTGCGAGCTGCCGCGCTCTATACCTGCGTGTGTCCCCTGG
AGTCCTTATCTTTTCCAACCTAACGATACGTGTATAGTGAGCGGCTGGGGCCGGGAGAAGGACAATGAACGA
GTTTTTTCCTTGCAATGGGGAGAAGTGAAGCTTATTTCCAATTGTTCAAAGTTTTATGGAAATAGATTTTAT
GAAAAAGAAATGGAGTGTGCGGGCACTTATGACGGGTCAATTGATGCTTGCAAAGGTGATAGCGGGGGCCCA
CTTGTCTGCATGGACGCTAACAACGTGACTTATGTGTGGGGTGTTGTGTCCTGGGGCGAAAACTGTGGCAAG
CCCGAGTTTCCCGGCGTATACACCAAAGTAGCTAATTATTTCGACTGGATTAGTTATCATGTTGGGCGGCCA
TTTATATCCCAGTATAATGTCTAA

(SEQ ID NO: 22)

RC133: CFI IDT – Manually optimised:

ATGAAGCTCCTGCACGTCTTCTTGCTGTTTCTCTGTTTCCACCTGAGATTTTGCAAAGTAACTTACACCAGT
CAAGAAGACTTGGTCGAGAAGAAATGTCTCGCCAAAAAGTATACTCACCTGAGCTGTGATAAAGTGTTCTGT
CAGCCGTGGCAGCGCTGCATTGAGGGTACATGTGTCTGTAAACTGCCTTATCAGTGTCCGAAGAACGGCACG
GCTGTCTGTGCTACTAACAGACGGTCTTTTCCTACTTATTGCCAGCAAAAGAGTTTGGAATGTCTCCACCCT
GGTACCAAGTTTCTCAACAATGGCACCTGTACTGCTGAAGGCAAATTCTCCGTCAGTCTCAAGCATGGTAAC
ACTGATTCTGAAGGGATAGTAGAAGTAAAGTTGGTTGACCAGGACAAGACGATGTTCATATGCAAGTCAAGC
TGGTCCATGCGCGAGGCGAATGTCGCTTGTCTTGATTTGGGCTTCCAGCAAGGGGCAGACACACAGAGAAGA
TTCAAATTGAGCGACCTGAGTATAAATTCAACCGAGTGCCTCCATGTACATTGCAGAGGGCTCGAGACTTCT
CTTGCTGAGTGTACATTTACGAAGAGGCGGACTATGGGATATCAGGACTTTGCTGACGTAGTGTGTTATACT
CAGAAAGCAGACAGTCCTATGGATGACTTTTTCCAATGCGTCAACGGCAAATACATCAGTCAAATGAAAGCG
TGCGACGGTATCAACGATTGTGGTGACCAGTCTGATGAGCTTTGCTGTAAAGCATGTCAAGGAAAGGGGTTC
CATTGCAAGAGTGGTGTATGTATTCCCTCACAATATCAGTGCAATGGGGAAGTCGATTGCATAACAGGCGAG
GATGAGGTGGGCTGCGCGGGATTTGCTTCTGTGGCGCAAGAGGAAACTGAGATCCTTACAGCGGATATGGAC
GCCGAACGAAGACGCATCAAATCTCTCCTTCCCAAACTTTCATGCGGCGTCAAAAACCGAATGCATATACGC
AGGAAGAGAATTGTTGGGGGAAAGCGGGCACAGCTGGGCGACCTCCCCTGGCAAGTTGCAATAAAGGATGCA
AGTGGGATAACGTGCGGGGGCATCTACATCGGGGGCTGCTGGATCTTGACTGCCGCTCACTGTCTTAGAGCC
TCTAAGACCCATAGATACCAAATCTGGACAACTGTAGTTGACTGGATACATCCGGACCTTAAACGCATAGTT
ATTGAATACGTTGACCGCATAATCTTTCATGAGAATTATAACGCGGGCACATACCAAAATGACATCGCCCTG
ATCGAGATGAAAAAGGACGGGAATAAAAAGGACTGCGAGCTGCCGCGCTCTATACCTGCGTGTGTCCCCTGG
AGTCCTTATCTTTTCCAACCTAACGATACGTGTATAGTGAGCGGCTGGGGCCGGGAGAAGGACAATGAACGA
GTTTTTTCCTTGCAATGGGGAGAAGTGAAGCTTATTTCCAATTGTTCAAAGTTTTATGGAAATAGATTTTAT
GAAAAAGAAATGGAGTGTGCGGGCACTTATGACGGGTCAATTGATGCTTGCAAAGGTGATAGCGGGGGCCCA
CTTGTCTGCATGGACGCTAACAATGTGACTTATGTGTGGGGTGTTGTGTCCTGGGGCGAAAACTGTGGCAAG
CCCGAGTTTCCCGGCGTATACACCAAAGTAGCTAATTATTTCGACTGGATTAGTTATCATGTTGGGCGGCCA
TTTATATCCCAGTATAATGTCTAA

(SEQ ID NO: 23)

RC134: CFI JCat – Basic:

ATGAAGCTGCTGCACGTGTTCCTGCTGTTCCTGTGCTTCCACCTGCGCTTCTGCAAGGTGACCTACACCAGC
CAGGAGGACCTGGTGGAGAAGAAGTGCCTGGCCAAGAAGTACACCCACCTGAGCTGCGACAAGGTGTTCTGC
CAGCCCTGGCAGCGCTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCCTACCAGTGCCCCAAGAACGGCACC
GCCGTGTGCGCCACCAACCGCCGCAGCTTCCCCACCTACTGCCAGCAGAAGAGCCTGGAGTGCCTGCACCCC
GGCACCAAGTTCCTGAACAACGGCACCTGCACCGCCGAGGGCAAGTTCAGCGTGAGCCTGAAGCACGGCAAC
ACCGACAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGACAAGACCATGTTCATCTGCAAGAGCAGC
TGGAGCATGCGCGAGGCCAACGTGGCCTGCCTGGACCTGGGCTTCCAGCAGGGCGCCGACACCCAGCGCCGC
TTCAAGCTGAGCGACCTGAGCATCAACAGCACCGAGTGCCTGCACGTGCACTGCCGCGGCCTGGAGACCAGC
CTGGCCGAGTGCACCTTCACCAAGCGCCGCACCATGGGCTACCAGGACTTCGCCGACGTGGTGTGCTACACC
CAGAAGGCCGACAGCCCCATGGACGACTTCTTCCAGTGCGTGAACGGCAAGTACATCAGCCAGATGAAGGCC
TGCGACGGCATCAACGACTGCGGCGACCAGAGCGACGAGCTGTGCTGCAAGGCCTGCCAGGGCAAGGGCTTC
CACTGCAAGAGCGGCGTGTGCATCCCCAGCCAGTACCAGTGCAACGGCGAGGTGGACTGCATCACCGGCGAG
GACGAGGTGGGCTGCGCCGGCTTCGCCAGCGTGGCCCAGGAGGAGACCGAGATCCTGACCGCCGACATGGAC
GCCGAGCGCCGCCGCATCAAGAGCCTGCTGCCCAAGCTGAGCTGCGGCGTGAAGAACCGCATGCACATCCGC
CGCAAGCGCATCGTGGGCGGCAAGCGCGCCCAGCTGGGCGACCTGCCCTGGCAGGTGGCCATCAAGGACGCC
AGCGGCATCACCTGCGGCGGCATCTACATCGGCGGCTGCTGGATCCTGACCGCCGCCCACTGCCTGCGCGCC
AGCAAGACCCACCGCTACCAGATCTGGACCACCGTGGTGGACTGGATCCACCCCGACCTGAAGCGCATCGTG
ATCGAGTACGTGGACCGCATCATCTTCCACGAGAACTACAACGCCGGCACCTACCAGAACGACATCGCCCTG
ATCGAGATGAAGAAGGACGGCAACAAGAAGGACTGCGAGCTGCCCCGCAGCATCCCCGCCTGCGTGCCCTGG
AGCCCCTACCTGTTCCAGCCCAACGACACCTGCATCGTGAGCGGCTGGGGCCGCGAGAAGGACAACGAGCGC
GTGTTCAGCCTGCAGTGGGGCGAGGTGAAGCTGATCAGCAACTGCAGCAAGTTCTACGGCAACCGCTTCTAC
GAGAAGGAGATGGAGTGCGCCGGCACCTACGACGGCAGCATCGACGCCTGCAAGGGCGACAGCGGCGGCCCC
CTGGTGTGCATGGACGCCAACAACGTGACCTACGTGTGGGGCGTGGTGAGCTGGGGCGAGAACTGCGGCAAG
CCCGAGTTCCCCGGCGTGTACACCAAGGTGGCCAACTACTTCGACTGGATCAGCTACCACGTGGGCCGCCCC
TTCATCAGCCAGTACAACGTGTAA

(SEQ ID NO: 24)

RC135: CFI JCat – Manually optimised:

ATGAAGCTGCTCCACGTGTTCCTGCTCTTCCTGTGCTTCCACCTGCGCTTCTGCAAGGTGACCTACACCAGC
CAGGAGGACCTGGTGGAGAAGAAATGCCTGGCCAAGAAATACACCCACCTGAGCTGCGACAAGGTGTTCTGC
CAGCCCTGGCAGCGCTGCATCGAGGGCACCTGCGTGTGCAAGCTGCCCTACCAGTGCCCCAAGAACGGCACC
GCCGTGTGCGCCACCAACCGCCGGAGCTTCCCCACCTACTGCCAGCAAAAGAGCCTGGAGTGCCTGCACCCC
GGCACCAAGTTCCTGAACAATGGCACCTGCACCGCCGAGGGCAAGTTCAGCGTGAGCCTGAAGCACGGCAAC
ACCGACAGCGAGGGCATCGTGGAGGTGAAGCTGGTGGACCAGGACAAGACCATGTTCATCTGCAAGAGCTCC
TGGAGCATGCGCGAGGCCAACGTGGCCTGCCTGGACCTGGGCTTCCAGCAAGGCGCCGACACCCAGCGCCGG
TTCAAGCTGAGCGACCTGAGCATCAACAGCACCGAGTGCCTGCACGTGCACTGCCGCGGCCTGGAGACCAGC
CTGGCCGAGTGCACCTTCACCAAGCGCCGGACCATGGGCTACCAGGACTTCGCCGACGTGGTCTGCTACACC
CAGAAGGCTGACTCTCCCATGGACGATTTCTTTCAGTGCGTGAACGGCAAGTACATCAGCCAGATGAAGGCC
TGCGACGGCATCAACGACTGCGGCGACCAGAGCGACGAGCTGTGCTGTAAGGCCTGCCAGGGCAAGGGCTTC
CACTGCAAGAGCGGCGTGTGCATCCCCAGCCAGTACCAGTGCAACGGCGAGGTGGACTGCATCACCGGCGAG
GACGAGGTGGGCTGCGCCGGCTTCGCCAGCGTGGCCCAGGAGGAAACCGAGATCCTGACCGCCGACATGGAC
GCCGAGCGCAGAAGGATCAAGAGCCTGCTCCCCAAGCTGAGCTGCGGCGTGAAGAACCGCATGCACATCCGC
AGAAAGCGCATCGTGGGCGGGAAGCGCGCCCAGCTGGGCGACCTGCCCTGGCAGGTGGCCATCAAGGACGCC
AGCGGCATCACCTGCGGCGGAATCTACATCGGCGGGTGCTGGATCCTGACCGCCGCTCACTGCCTGCGCGCC
AGCAAGACCCACCGCTACCAGATCTGGACCACAGTGGTCGACTGGATCCACCCCGACCTGAAGCGCATCGTG
ATCGAGTACGTGGACCGCATCATTTTCCACGAGAACTACAACGCCGGCACCTACCAGAACGACATCGCCCTG
ATCGAGATGAAGAAAGATGGAAACAAGAAAGACTGCGAGCTGCCCCGCAGCATCCCCGCCTGCGTGCCCTGG
AGCCCCTACCTGTTCCAGCCCAACGACACCTGCATCGTGAGCGGCTGGGGCCGCGAGAAGGACAACGAGCGC
GTGTTCAGCCTGCAGTGGGGCGAGGTGAAGCTGATCAGCAACTGCAGCAAGTTCTACGGCAACCGCTTCTAC
GAGAAGGAGATGGAGTGCGCCGGCACCTACGACGGCAGCATCGACGCCTGCAAGGGCGACAGCGGCGGGCCC
CTGGTGTGCATGGACGCCAACAATGTGACCTACGTGTGGGGCGTGGTCAGCTGGGGCGAGAACTGCGGCAAG
CCCGAGTTCCCCGGCGTGTACACCAAGGTGGCCAACTACTTCGACTGGATCAGCTACCACGTGGGCCGCCCC
TTTATCTCTCAATACAACGTCTAA

(SEQ ID NO: 25)

RC136: CFI COOL – Basic: see SEQ ID NO: 10, above.

RC137: CFI COOL – Manually optimised:

ATGAAACTGCTCCATGTCTTCCTCCTGTTCCTGTGCTTCCACCTCCGTTTCTGTAAAGTCACCTACACTAGC
CAGGAGGATCTGGTGGAGAAGAAATGCCTGGCCAAGAAATATACCCACCTGAGCTGCGACAAAGTGTTCTGC
CAGCCCTGGCAACGCTGCATTGAAGGCACTTGTGTGTGCAAGCTGCCCTACCAGTGCCCCAAGAACGGCACG
GCCGTGTGTGCCACCAACAGGAGAAGCTTCCCCACCTACTGCCAGCAAAAGAGCCTGGAATGCCTCCACCCT
GGCACCAAGTTTCTGAACAATGGGACCTGCACAGCCGAGGGGAAATTCAGCGTCTCCCTCAAGCACGGCAAT
ACAGACTCCGAGGGCATTGTGGAAGTGAAGCTGGTGGACCAGGACAAGACCATGTTCATCTGCAAAAGCTCC
TGGTCCATGCGGGAGGCCAATGTCGCCTGCCTGGACCTGGGCTTCCAGCAAGGCGCTGATACACAGCGCAGA
TTTAAACTCAGTGACCTCAGCATCAACAGCACTGAGTGTCTGCACGTGCACTGCCGGGGCCTGGAGACCAGC
CTGGCTGAGTGCACCTTCACCAAGCGCAGGACCATGGGCTACCAGGATTTTGCAGATGTGGTCTGCTACACC
CAGAAGGCAGACAGCCCCATGGATGACTTCTTTCAGTGTGTCAATGGCAAGTACATTTCCCAGATGAAGGCT
TGTGACGGGATCAATGATTGCGGGGATCAGAGCGATGAGCTCTGCTGTAAGGCCTGCCAAGGGAAGGGCTTT
CACTGCAAGTCTGGGGTGTGCATCCCTTCTCAGTATCAGTGCAACGGAGAGGTGGACTGCATCACTGGGGAG
GACGAGGTGGGCTGTGCTGGCTTCGCCTCTGTGGCCCAGGAGGAAACAGAGATCCTCACAGCTGACATGGAT
GCAGAGCGGAGGCGCATCAAGAGTCTGCTCCCAAAGCTCTCCTGCGGCGTTAAGAATCGCATGCACATCCGG
AGGAAGCGGATCGTTGGAGGCAAACGGGCTCAGCTGGGGGACTTGCCGTGGCAGGTGGCCATCAAAGATGCC
TCCGGAATCACCTGTGGTGGCATCTACATCGGCGGGTGCTGGATCCTGACCGCCGCTCACTGCCTTCGGGCC
AGCAAGACCCATCGCTACCAGATCTGGACCACAGTGGTCGATTGGATTCACCCCGACCTGAAGAGGATTGTC
ATTGAGTATGTCGACCGCATCATTTTCCATGAAAACTACAATGCCGGGACGTATCAGAACGACATCGCCCTC
ATCGAGATGAAGAAAGATGGGAACAAGAAAGACTGTGAGCTGCCTCGCTCCATCCCCGCCTGTGTACCATGG
TCTCCGTACCTGTTCCAGCCAAATGACACATGCATCGTGAGCGGCTGGGGCCGCGAGAAAGACAACGAGAGG
GTCTTCTCCCTGCAGTGGGGTGAAGTCAAGCTGATCAGCAACTGCTCCAAGTTCTACGGCAACCGCTTCTAT
GAGAAGGAGATGGAGTGCGCCGGCACCTATGACGGCAGCATTGACGCGTGCAAGGGAGACAGTGGGGGCCCC
CTGGTCTGCATGGACGCCAACAATGTGACCTACGTGTGGGGAGTTGTGTCCTGGGGCGAGAACTGTGGCAAG
CCTGAGTTCCCGGGCGTGTACACAAAGGTGGCAAACTATTTTGACTGGATCTCCTATCACGTTGGCAGGCCC
TTCATTAGCCAGTATAATGTATAA

(SEQ ID NO: 26)

RC138: FHL-1 GeneArt – Basic:

ATGAGACTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCCGAGGATTGCAATGAGCTG
CCTCCTCGGAGAAACACCGAGATCCTGACAGGCTCTTGGAGCGACCAGACATACCCTGAGGGAACCCAGGCC
ATCTACAAGTGCAGACCCGGCTACAGAAGCCTGGGCAACATCATCATGGTCTGCCGGAAAGGCGAGTGGGTC
GCCCTGAATCCTCTGCGGAAGTGCCAGAAAAGACCCTGCGGACACCCTGGCGATACCCCTTTCGGAACCTTT
ACACTGACCGGCGGCAACGTGTTCGAGTACGGCGTGAAAGCCGTGTACACCTGTAACGAGGGCTACCAGCTG
CTGGGCGAGATCAACTACAGAGAGTGCGATACCGACGGCTGGACCAACGACATCCCTATCTGCGAGGTGGTC
AAGTGCCTGCCTGTGACAGCCCCTGAGAACGGCAAGATTGTGTCCAGCGCCATGGAACCCGACAGAGAGTAC
CACTTTGGCCAGGCCGTCAGATTCGTGTGCAACAGCGGCTACAAGATCGAGGGCGACGAGGAAATGCACTGC
AGCGACGATGGCTTCTGGTCCAAAGAAAAGCCTAAGTGCGTGGAAATCAGCTGCAAGAGCCCCGACGTGATC
AACGGCAGCCCTATCAGCCAGAAGATTATCTACAAAGAGAACGAGCGGTTCCAGTACAAGTGTAACATGGGC
TACGAGTACAGCGAGAGGGGCGACGCCGTGTGTACAGAATCTGGATGGCGACCTCTGCCTAGCTGCGAGGAA
AAGAGCTGCGACAACCCTTACATCCCCAACGGCGACTACAGCCCACTGCGGATCAAACACAGAACCGGCGAC
GAGATCACCTACCAGTGCCGGAATGGCTTCTACCCTGCCACCAGAGGCAATACCGCCAAGTGTACAAGCACC
GGCTGGATCCCTGCTCCTCGGTGTACACTGAAGCCCTGCGACTACCCCGATATCAAGCACGGCGGACTGTAC
CACGAGAACATGCGGAGGCCTTACTTCCCTGTGGCCGTGGGCAAGTACTACAGCTACTACTGCGACGAGCAC
TTCGAGACACCCAGCGGCAGCTACTGGGATCACATCCACTGTACCCAGGACGGCTGGTCACCAGCTGTGCCT
TGCCTGAGAAAGTGCTACTTCCCCTACCTGGAAAACGGCTACAACCAGAACTACGGCCGGAAGTTCGTGCAG
GGCAAGAGCATCGATGTGGCCTGCCATCCTGGATACGCCCTGCCTAAGGCTCAGACCACCGTGACCTGCATG
GAAAATGGCTGGTCCCCAACACCTCGGTGCATCCGGGTGTCCTTCACACTGTAA

(SEQ ID NO: 27)

RC139: FHL-1 GeneArt – Manually optimised:

ATGAGACTGCTCGCCAAGATCATTTGCCTGATGCTGTGGGCCATCTGCGTGGCCGAGGATTGCAATGAGCTG
CCTCCCCGGAGAAACACCGAGATCCTGACAGGCTCTTGGAGCGACCAGACATACCCTGAGGGAACCCAGGCC
ATCTACAAGTGCAGACCCGGCTACAGAAGCCTGGGCAACATCATTATGGTCTGCCGGAAAGGCGAGTGGGTC
GCCCTGAATCCTCTGCGGAAGTGCCAGAAAAGACCCTGCGGACACCCTGGCGATACCCCTTTCGGAACCTTT
ACACTGACCGGCGGGAACGTGTTCGAGTACGGCGTGAAAGCCGTGTACACCTGTAACGAGGGCTACCAGCTG
CTCGGCGAGATCAACTACAGAGAGTGCGATACCGACGGCTGGACCAACGACATCCCTATCTGCGAGGTGGTC
AAGTGCCTGCCTGTGACAGCCCCTGAGAACGGCAAGATTGTGTCCAGCGCCATGGAACCCGACAGAGAGTAC
CACTTTGGCCAGGCCGTCAGATTCGTGTGCAACAGCGGCTACAAGATCGAGGGCGACGAGGAAATGCACTGC
AGCGACGATGGCTTCTGGTCCAAAGAAAAGCCTAAGTGCGTGGAAATCAGCTGCAAGAGCCCCGACGTGATC
AACGGCAGCCCTATCAGCCAGAAGATTATCTACAAAGAGAACGAGCGGTTCCAGTACAAGTGTAACATGGGC
TACGAGTACAGCGAGAGGGGCGACGCCGTGTGTACAGAATCTGGATGGCGACCTCTGCCTAGCTGCGAGGAA
AAGAGCTGCGACAACCCTTACATCCCCAACGGCGACTACAGCCCACTGCGGATCAAACACAGAACCGGCGAC
GAGATCACCTACCAGTGCCGGAATGGCTTCTACCCTGCCACCAGAGGCAATACCGCCAAGTGTACAAGCACC
GGCTGGATCCCTGCTCCTCGGTGTACACTGAAGCCCTGCGACTACCCCGATATCAAGCACGGCGGACTGTAC
CACGAGAACATGCGGAGGCCTTACTTCCCTGTGGCCGTGGGCAAGTACTATAGCTACTATTGCGACGAGCAC
TTCGAGACACCCAGCGGCAGCTACTGGGATCACATCCACTGTACCCAGGACGGCTGGTCACCAGCTGTGCCT
TGCCTGAGAAAGTGCTACTTCCCCTACCTGGAAAACGGCTACAACCAGAACTACGGCCGGAAGTTCGTGCAG
GGCAAGAGCATCGATGTGGCCTGCCATCCTGGATACGCCCTGCCTAAGGCTCAGACCACAGTGACCTGCATG
GAAAATGGCTGGTCCCCAACACCTCGGTGCATCCGGGTGTCCTTCACACTGTAA

(SEQ ID NO: 28)

RC140: FHL-1 Genscript – Basic:

ATGCGGCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCCGAGGACTGTAACGAGCTG
CCCCCTCGGAGAAATACAGAGATCCTGACCGGCTCTTGGAGCGATCAGACATATCCTGAGGGCACCCAGGCC
ATCTACAAGTGCAGGCCAGGCTATCGCTCCCTGGGCAACATCATCATGGTGTGCAGGAAGGGAGAGTGGGTG
GCCCTGAATCCTCTGAGGAAGTGCCAGAAGAGGCCATGTGGACACCCAGGCGACACCCCTTTCGGCACCTTT
ACACTGACCGGCGGCAACGTGTTCGAGTACGGCGTGAAGGCCGTGTATACATGCAACGAGGGCTACCAGCTG
CTGGGCGAGATCAATTACAGAGAGTGTGACACAGATGGCTGGACCAACGATATCCCAATCTGCGAGGTGGTG
AAGTGTCTGCCAGTGACCGCCCCCGAGAATGGCAAGATCGTGAGCTCCGCCATGGAGCCCGACAGGGAGTAT
CACTTCGGCCAGGCCGTGCGCTTCGTGTGCAACTCTGGCTACAAGATCGAGGGCGATGAGGAGATGCACTGT
AGCGACGATGGCTTCTGGTCCAAGGAGAAGCCCAAGTGCGTGGAGATCAGCTGTAAGTCCCCAGACGTGATC
AATGGCTCTCCCATCAGCCAGAAGATCATCTATAAGGAGAACGAGAGGTTTCAGTACAAGTGCAATATGGGC
TACGAGTATTCCGAGAGGGGCGATGCCGTGTGCACCGAGTCTGGCTGGAGACCACTGCCCTCCTGCGAGGAG
AAGTCTTGTGACAACCCATATATCCCCAATGGCGATTACTCTCCCCTGCGGATCAAGCACAGAACAGGCGAC
GAGATCACCTATCAGTGCCGGAACGGCTTCTACCCTGCCACAAGAGGCAATACCGCCAAGTGTACAAGCACC
GGATGGATCCCTGCACCAAGGTGCACCCTGAAGCCTTGTGACTATCCAGATATCAAGCACGGCGGCCTGTAT
CACGAGAATATGAGGCGCCCTTACTTCCCAGTGGCCGTGGGCAAGTACTATAGCTACTATTGCGACGAGCAC
TTTGAGACCCCTTCCGGCTCTTACTGGGACCACATCCACTGTACACAGGATGGATGGTCCCCAGCAGTGCCT
TGCCTGAGGAAGTGTTACTTCCCATATCTGGAGAACGGCTACAACCAGAATTATGGCCGCAAGTTTGTGCAG
GGCAAGAGCATCGATGTGGCATGCCACCCAGGATACGCACTGCCTAAGGCACAGACCACAGTGACATGCATG
GAGAATGGCTGGTCTCCCACCCCTCGGTGTATCAGAGTGAGCTTTACACTGTGA

(SEQ ID NO: 29)

RC141: FHL-1 Genscript – Manually optimised:

ATGCGGCTGCTCGCCAAGATCATTTGCCTGATGCTGTGGGCCATCTGCGTGGCCGAGGACTGTAACGAGCTG
CCCCCTCGGAGAAATACAGAGATCCTGACCGGCTCTTGGAGCGATCAGACATATCCTGAGGGCACCCAGGCC
ATCTACAAGTGCAGGCCAGGCTATCGCTCCCTGGGCAACATCATTATGGTGTGCAGGAAGGGAGAGTGGGTG
GCCCTGAATCCTCTGAGGAAGTGCCAGAAGAGGCCATGTGGACACCCAGGCGACACCCCTTTCGGCACCTTT
ACACTGACCGGCGGGAACGTGTTCGAGTACGGCGTGAAGGCCGTGTATACATGCAACGAGGGCTACCAGCTG
CTCGGCGAGATCAATTACAGAGAGTGTGACACAGATGGCTGGACCAACGATATCCCAATCTGCGAGGTGGTC
AAGTGTCTGCCAGTGACCGCCCCCGAGAATGGCAAGATCGTGAGCTCCGCCATGGAGCCCGACAGGGAGTAT
CACTTCGGCCAGGCCGTGCGCTTCGTGTGCAACTCTGGCTACAAGATCGAGGGCGATGAGGAAATGCACTGT

AGCGACGATGGCTTCTGGTCCAAGGAGAAGCCCAAGTGCGTGGAGATCAGCTGCAAGTCCCCAGACGTGATC
AATGGCTCTCCCATCAGCCAGAAGATCATTTATAAGGAGAACGAGAGGTTTCAGTACAAGTGCAATATGGGC
TACGAGTATTCCGAGAGGGGCGATGCCGTGTGCACCGAGTCTGGCTGGAGACCACTGCCCTCCTGCGAGGAA
AAGTCTTGTGACAACCCATATATCCCCAATGGCGATTACTCTCCCCTGCGGATCAAGCACAGAACAGGCGAC
GAGATCACCTATCAGTGCCGGAACGGCTTCTACCCTGCCACAAGAGGCAATACCGCCAAGTGTACAAGCACC
GGATGGATCCCTGCACCAAGGTGCACCCTGAAGCCTTGTGACTATCCAGATATCAAGCACGGCGGGCTGTAT
CACGAGAATATGAGGCGCCCTTACTTCCCAGTGGCCGTGGGCAAGTACTATAGCTACTATTGCGACGAGCAC
TTTGAGACCCCTTCCGGCTCTTACTGGGACCACATCCACTGTACACAGGATGGATGGTCCCCAGCAGTGCCT
TGCCTGAGGAAGTGTTACTTCCCATATCTGGAGAACGGCTACAACCAGAATTATGGCCGCAAGTTTGTGCAG
GGCAAGAGCATCGATGTGGCATGCCACCCAGGATACGCACTGCCTAAGGCACAGACCACAGTGACATGCATG
GAGAATGGCTGGTCTCCCACCCCTCGGTGTATCAGAGTGAGCTTTACACTGTGA

(SEQ ID NO: 30)

RC142: FHL-1 IDT – Basic:

ATGAGACTGCTTGCGAAAATTATATGCCTGATGCTTTGGGCTATTTGCGTTGCGGAAGACTGTAACGAACTC
CCGCCCCGCCGAAATACAGAGATCCTCACAGGCAGTTGGAGCGACCAAACGTACCCTGAAGGTACGCAGGCC
ATATATAAGTGTAGGCCAGGCTACAGATCACTTGGTAACATAATAATGGTATGTCGGAAAGGAGAGTGGGTC
GCTCTCAACCCTCTTAGGAAATGTCAAAAAAGACCCTGTGGGCATCCGGGAGATACGCCTTTCGGGACATTC
ACTCTCACGGGCGGAAACGTATTCGAATATGGCGTGAAGGCAGTGTATACCTGCAATGAAGGGTATCAGCTG
CTTGGGGAAATTAATTATAGGGAATGTGACACGGATGGGTGGACAAACGATATTCCAATATGCGAAGTAGTT
AAATGCCTGCCCGTTACTGCACCGGAGAATGGCAAAATAGTCAGTAGTGCAATGGAGCCGGATCGCGAGTAT
CATTTTGGTCAGGCCGTGCGGTTCGTATGTAATTCTGGGTACAAGATCGAAGGTGACGAAGAGATGCATTGC
TCAGATGACGGCTTTTGGAGTAAAGAAAAGCCTAAGTGTGTTGAAATCAGCTGTAAGAGTCCAGACGTGATT
AACGGTTCCCCGATCTCTCAGAAGATAATTTACAAGGAAAACGAACGATTCCAATATAAGTGTAACATGGGC
TACGAGTATTCCGAGCGAGGTGACGCAGTATGTACGGAAAGCGGGTGGCGACCTCTGCCCTCCTGCGAGGAA
AAGAGCTGTGATAATCCGTATATCCCCAACGGTGACTATAGCCCACTGCGCATAAAACATCGGACGGGAGAT
GAGATTACATACCAATGCCGCAATGGTTTTTACCCCGCCACCCGAGGGAACACGGCAAAGTGCACTTCTACG
GGGTGGATTCCAGCTCCTAGGTGCACTCTTAAACCCTGCGACTACCCAGATATCAAGCATGGTGGACTGTAT
CATGAGAATATGAGGAGACCATACTTTCCAGTTGCAGTGGGCAAGTACTATAGCTATTACTGTGATGAGCAC
TTTGAAACTCCGTCTGGGAGCTACTGGGATCATATCCATTGTACGCAAGACGGCTGGAGTCCAGCAGTTCCA
TGCTTGCGGAAATGTTATTTTCCCTACCTCGAAAACGGATATAATCAGAACTATGGGAGGAAGTTTGTTCAA
GGCAAAAGCATTGATGTGGCATGTCACCCCGGTTATGCCCTGCCCAAGGCGCAAACCACAGTAACTTGCATG
GAGAATGGATGGAGCCCCACACCCAGATGTATACGAGTATCCTTCACGCTTTGA

(SEQ ID NO: 31)

RC143: FHL-1 IDT – Manually optimised:

ATGAGACTGCTTGCGAAAATTATATGCCTGATGCTTTGGGCTATTTGCGTTGCGGAAGACTGTAACGAACTC
CCGCCCCGCCGAAATACAGAGATCCTCACAGGCAGTTGGAGCGACCAAACGTACCCTGAAGGCACGCAGGCC
ATATATAAGTGTAGGCCAGGCTACAGATCACTTGGTAACATAATCATGGTGTGTCGGAAAGGAGAGTGGGTC
GCTCTCAACCCTCTTCGCAAATGTCAAAAAAGACCCTGTGGGCATCCGGGAGATACGCCTTTCGGGACATTC
ACTCTCACGGGCGGAAACGTATTCGAATATGGCGTGAAGGCAGTGTATACCTGCAATGAAGGGTATCAGCTG
CTTGGGGAAATTAATTATAGGGAATGTGACACGGATGGGTGGACAAACGATATTCCAATATGCGAAGTAGTT
AAATGCCTGCCCGTTACTGCACCGGAGAATGGCAAAATAGTCAGTAGCGCAATGGAGCCGGATCGCGAGTAT
CATTTTGGTCAGGCCGTGCGGTTCGTATGTAATTCTGGGTACAAGATCGAAGGTGACGAAGAGATGCATTGC
TCAGATGACGGCTTTTGGAGCAAGGAAAAGCCTAAGTGTGTTGAAATCAGCTGTAAGAGTCCAGACGTGATT
AACGGTTCCCCGATCTCTCAGAAGATAATTTACAAGGAAAACGAACGATTCCAATATAAGTGTAACATGGGC
TACGAGTATTCCGAGCGAGGTGACGCAGTATGTACGGAAAGCGGGTGGCGACCTCTGCCCTCCTGCGAGGAA
AAGAGCTGTGATAATCCGTATATCCCCAACGGCGACTATAGCCCACTGCGCATAAAACATCGGACGGGAGAT
GAGATTACATACCAATGCCGCAATGGTTTTTACCCCGCCACCCGAGGGAACACGGCAAAGTGCACTTCTACG
GGGTGGATTCCAGCTCCTAGGTGCACTCTTAAACCCTGCGACTACCCAGATATCAAGCATGGTGGACTGTAT
CATGAGAATATGAGGAGACCATACTTTCCAGTTGCAGTGGGCAAGTACTATAGCTATTACTGTGATGAGCAC
TTTGAAACTCCGTCTGGGAGCTACTGGGATCATATCCATTGTACGCAAGACGGCTGGAGTCCAGCAGTTCCA
TGCTTGCGGAAATGTTATTTTCCCTACCTCGAAAACGGATATAATCAGAATTACGGCAGGAAATTTGTGCAA
GGCAAAAGCATTGATGTGGCATGTCACCCCGGTTATGCCCTGCCCAAGGCGCAAACCACAGTAACTTGCATG
GAGAATGGATGGAGCCCCACACCCAGATGTATACGAGTATCCTTCACGCTTTGA

(SEQ ID NO: 32)

RC144: FHL-1 JCat – Basic

ATGCGCCTGCTGGCCAAGATCATCTGCCTGATGCTGTGGGCCATCTGCGTGGCCGAGGACTGCAACGAGCTG
CCCCCCCGCCGCAACACCGAGATCCTGACCGGCAGCTGGAGCGACCAGACCTACCCCGAGGGCACCCAGGCC
ATCTACAAGTGCCGCCCCGGCTACCGCAGCCTGGGCAACATCATCATGGTGTGCCGCAAGGGCGAGTGGGTG
GCCCTGAACCCCCTGCGCAAGTGCCAGAAGCGCCCCTGCGGCCACCCCGGCGACACCCCCTTCGGCACCTTC
ACCCTGACCGGCGGCAACGTGTTCGAGTACGGCGTGAAGGCCGTGTACACCTGCAACGAGGGCTACCAGCTG
CTGGGCGAGATCAACTACCGCGAGTGCGACACCGACGGCTGGACCAACGACATCCCCATCTGCGAGGTGGTG
AAGTGCCTGCCCGTGACCGCCCCCGAGAACGGCAAGATCGTGAGCAGCGCCATGGAGCCCGACCGCGAGTAC
CACTTCGGCCAGGCCGTGCGCTTCGTGTGCAACAGCGGCTACAAGATCGAGGGCGACGAGGAGATGCACTGC
AGCGACGACGGCTTCTGGAGCAAGGAGAAGCCCAAGTGCGTGGAGATCAGCTGCAAGAGCCCCGACGTGATC
AACGGCAGCCCCATCAGCCAGAAGATCATCTACAAGGAGAACGAGCGCTTCCAGTACAAGTGCAACATGGGC
TACGAGTACAGCGAGCGCGGCGACGCCGTGTGCACCGAGAGCGGCTGGCGCCCCCTGCCCAGCTGCGAGGAG
AAGAGCTGCGACAACCCCTACATCCCCAACGGCGACTACAGCCCCCTGCGCATCAAGCACCGCACCGGCGAC
GAGATCACCTACCAGTGCCGCAACGGCTTCTACCCCGCCACCCGCGGCAACACCGCCAAGTGCACCAGCACC
GGCTGGATCCCCGCCCCCCGCTGCACCCTGAAGCCCTGCGACTACCCCGACATCAAGCACGGCGGCCTGTAC
CACGAGAACATGCGCCGCCCCTACTTCCCCGTGGCCGTGGGCAAGTACTACAGCTACTACTGCGACGAGCAC
TTCGAGACCCCCAGCGGCAGCTACTGGGACCACATCCACTGCACCCAGGACGGCTGGAGCCCCGCCGTGCCC
TGCCTGCGCAAGTGCTACTTCCCCTACCTGGAGAACGGCTACAACCAGAACTACGGCCGCAAGTTCGTGCAG
GGCAAGAGCATCGACGTGGCCTGCCACCCCGGCTACGCCCTGCCCAAGGCCCAGACCACCGTGACCTGCATG
GAGAACGGCTGGAGCCCCACCCCCCGCTGCATCCGCGTGAGCTTCACCCTGTAA

(SEQ ID NO: 33)

RC145: FHL-1 JCat – Manually optimised

ATGCGCCTGCTCGCCAAGATCATTTGCCTGATGCTGTGGGCCATCTGCGTGGCCGAGGACTGCAACGAGCTG
CCCCCTCGCCGGAACACCGAGATCCTGACCGGCAGCTGGAGCGACCAGACCTACCCCGAGGGCACCCAGGCC
ATCTACAAGTGCCGCCCCGGCTACCGCAGCCTGGGCAACATCATTATGGTGTGCCGCAAGGGCGAGTGGGTG
GCCCTGAACCCCCTGCGCAAGTGCCAGAAGCGCCCCTGCGGCCACCCCGGCGACACCCCCTTCGGCACCTTC
ACCCTGACCGGCGGCAACGTGTTCGAGTACGGCGTGAAGGCCGTGTACACCTGCAACGAGGGCTACCAGCTG
CTCGGCGAGATCAACTACCGCGAGTGCGACACCGACGGCTGGACCAACGACATCCCCATCTGCGAGGTGGTC
AAGTGCCTGCCCGTGACCGCCCCCGAGAACGGCAAGATCGTGAGCTCCGCCATGGAGCCCGACCGCGAGTAC
CACTTCGGCCAGGCCGTGCGCTTCGTGTGCAACAGCGGCTACAAGATCGAGGGCGACGAGGAGATGCACTGC
AGCGACGATGGCTTCTGGAGCAAGGAGAAGCCCAAGTGCGTGGAGATCAGCTGCAAGAGCCCCGACGTGATC
AACGGCAGCCCCATCAGCCAGAAGATCATTTACAAGGAGAACGAGCGCTTCCAGTACAAGTGCAACATGGGC
TACGAGTACAGCGAGCGCGGCGACGCCGTGTGCACCGAGAGCGGCTGGCGCCCCCTGCCCAGCTGCGAGGAA
AAGAGCTGCGACAACCCCTACATCCCCAACGGCGACTACAGCCCCCTGCGCATCAAGCACCGCACCGGCGAC
GAGATCACCTACCAGTGCCGCAACGGCTTCTACCCCGCCACCCGCGGCAACACCGCCAAGTGCACCAGCACC
GGCTGGATCCCCGCCCCCCGCTGCACCCTGAAGCCCTGCGACTACCCCGACATCAAGCACGGCGGGCTGTAC
CACGAGAACATGCGCCGGCCCTACTTCCCCGTGGCCGTGGGCAAGTACTATAGCTACTATTGCGACGAGCAC
TTCGAGACCCCCAGCGGCAGCTACTGGGACCACATCCACTGCACCCAGGACGGCTGGAGCCCCGCCGTGCCC
TGCCTGCGCAAGTGCTACTTCCCCTACCTGGAGAACGGCTACAACCAGAACTACGGCCGCAAGTTCGTGCAG
GGCAAGAGCATCGACGTGGCCTGCCACCCCGGCTACGCCCTGCCCAAGGCCCAGACCACAGTGACCTGCATG
GAGAACGGCTGGAGCCCCACCCCCCGCTGCATCCGCGTGAGCTTCACCCTGTAA

(SEQ ID NO: 34)

RC146: FHL-1 COOL – Basic: see SEQ ID NO: 12, above.

RC147: FHL-1 COOL – Manually optimised:

ATGCGCCTCCTGGCCAAGATCATTTGCCTCATGCTGTGGGCCATCTGCGTGGCTGAGGACTGCAATGAGCTG
CCGCCCAGGAGAAACACAGAGATCCTGACAGGGAGCTGGTCTGACCAGACCTACCCTGAGGGCACCCAGGCG
ATCTACAAGTGCCGGCCGGGCTACAGGAGCCTGGGGAACATCATTATGGTGTGTAGAAAGGGCGAATGGGTG

GCCCTCAACCCCCTGAGGAAGTGCCAGAAGCGGCCCTGTGGCCACCCCGGGGACACACCCTTCGGGACCTTC
ACCCTGACCGGCGGGAATGTGTTTGAGTACGGCGTGAAGGCTGTCTACACATGCAACGAGGGGTACCAGCTG
CTCGGCGAGATTAACTACCGGGAGTGTGACACCGATGGGTGGACCAACGACATTCCCATCTGTGAGGTGGTC
AAGTGTCTCCCCGTGACAGCCCCAGAAAATGGCAAAATTGTGAGCTCCGCCATGGAGCCTGACCGCGAATAT
CACTTTGGGCAGGCCGTGAGGTTTGTGTGCAACTCGGGCTACAAAATTGAAGGTGATGAGGAAATGCACTGC
AGCGATGACGGCTTCTGGTCCAAGGAGAAGCCCAAATGTGTGGAGATCTCCTGCAAGTCTCCCGACGTGATC
AACGGCAGCCCAATCAGCCAGAAGATTATCTACAAAGAGAACGAGCGCTTCCAGTACAAGTGTAACATGGGC
TATGAGTATTCAGAGAGGGGAGATGCCGTCTGCACTGAGAGCGGCTGGAGACCACTGCCTAGCTGCGAGGAA
AAGAGTTGTGACAACCCTTACATCCCAAATGGCGACTACTCCCCTCTGCGGATCAAACACCGGACCGGGGAT
GAAATCACCTATCAGTGCCGCAATGGATTCTACCCGGCCACCCGCGGCAACACCGCCAAATGCACCAGCACA
GGCTGGATCCCCGCCCCCCGCTGTACGCTGAAGCCTTGCGACTATCCAGACATCAAGCACGGAGGCCTGTAC
CACGAAAACATGCGGAGGCCTTATTTCCCTGTGGCAGTGGGGAAGTACTATAGCTACTATTGCGACGAGCAC
TTCGAGACCCCCTCTGGCTCCTACTGGGACCACATCCACTGCACACAGGACGGCTGGTCTCCAGCTGTGCCC
TGCCTGAGGAAATGCTACTTCCCCTACCTGGAGAACGGATACAACCAGAACTATGGCCGCAAGTTCGTGCAG
GGCAAGAGCATCGATGTGGCCTGCCACCCTGGCTACGCCCTGCCCAAGGCCCAGACAACTGTGACCTGCATG
GAGAATGGTTGGAGCCCCACCCCGCGCTGCATCCGGGTGTCCTTCACGCTCTGA

(SEQ ID NO: 35)

Generation of plasmids

All 10 codon optimised sequences were synthesised and cloned by them into an AAV vector backbone. The vector also comprised AAV-2 left and right inverted terminal repeats (ITRs), which flank a modified CBA/CAG promoter (chicken beta-actin with CMV enhancer; “CBA”). The promoter drives expression of the codon optimised FHL1 or CFI. In addition, the transgene was followed downstream by a modified Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) sequence and a Bovine growth hormone poly-A (bGH polyA) sequence provided 3’ to the cDNA.

Transfection

All 20 plasmids were transfected into ARPE19 cells using the following procedure:

Day 1: ARPE19 cells were dissociated and counted using a ViCell. Cells were seeded in a 48 well plate at 6×10^4 cells per well in 500 µL DMEM, 10% FBS per well.

Day 2: Confluency was checked and was found to be between 70-80%. Cells were then transfected with 0.25 µg plasmid DNA using PEI at a 1:3 DNA:PEI ratio in duplicate:

1. 2× 0.25 µg DNA was diluted in 2× 5 µL PBS.
2. 2× 0.75 µL PEI was diluted in 2× 5 µL PBS.
3. PEI mix was added dropwise to DNA mix, mixed and then incubated at room temperature for 20 minutes.
4. 2× 250 µL DMEM/Glutamax/10% FBS was added to the mixture.

5. Media was removed from the cells and replaced with 250 µL DNA/PEI complexes per well.

Day 3: Media was removed and replaced with 125 µL serum-free DMEM/Glutamax.

Day 5: Media was harvested, centrifuged at 14000 rpm for 10 minutes at 4°C then supernatant was transferred to a fresh tube.

Western blot

Supernatants from the transfection were analysed by Western blot (primary antibodies to CFI and FHL-1: goat antiserum CFI 1:3000; Quidel A312 1:3000; and secondary antibody rabbit anti-goat HRP 1:5000 were used).

The Western blot analyses are shown in Figure 2.

CFI ELISA

Supernatants from the transfection were analysed by ELISA for CFI using the following procedure:

Day 1: An ELISA plate was coated with 50 µL per well sheep anti-CFI polyclonal antibody diluted 1:4000 in 1x coating buffer. Plates stored at 4°C overnight.

Day 2: The plate was washed 3 times with 200 µL per well PBS-Tween (0.05%) then blotted on a tissue. 200 µL 1% BSA fraction V in PBS-Tween (0.05%) was applied to each well and allowed to block for 2 hours at room temperature.

Samples and standard curve were prepared during the blocking incubation. A standard curve was made from purified CFI protein (Sigma C5938-1MG) diluted into DMEM 2% FBS. Samples were diluted 1:10, 1:20 and 1:40 in DMEM 2% FBS.

After 2 hours blocking, the plate was washed 3 times, as described above, then 50 µL sample or standard was loaded onto each well and incubated at room temperature for 1 hour.

After 1 hour the plate was washed as above, then anti-CFI (Ox21) antibody was diluted 1:2000 in DMEM 5% FBS and 50 µL was applied to each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above then donkey anti-mouse-HRP antibody was diluted 1:5000 in DMEM 5% FBS, and 50 µL was applied to each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above, then 100 µL TMB reagent was applied to each well and incubated at room temperature in the dark for approximately 15 minutes. Once sufficient blue colour had been obtained, 100 µL 1 M sulphuric acid was added to each well to stop the reaction.

The A450 was then recorded with and data were processed and transferred to Microsoft Excel for analysis.

The CFI ELISA results are shown in Figure 3.

FHL1 ELISA

Supernatants from the transfection were analysed by ELISA for FHL1 using the following procedure:

Day 1: An ELISA plate was coated with 50 µL per well anti-FHL-1 antibody (Biorad, AbD33594.1) diluted to 5 µg/mL in 100 mM Carbonate/Bicarbonate buffer, pH 9.6. Plates were stored at 4°C overnight.

Day 2: The plate was washed 3 times with 200 µL per well PBS-Tween (0.05%) then blotted on a tissue. 200 µL 1% BSA fraction V in PBS-Tween (0.05%) was applied to each well and allowed to block for 2 hours at room temperature.

Samples and standard curve were prepared during the blocking incubation. A standard curve was made from FHL1-His protein diluted with DMEM + 2% FBS. Samples were diluted 1:5, 1:10 and 1:30 in blocking solution.

After 2 hours blocking, the plate was washed 3 times, as described above then 50 µL sample or standard was loaded onto each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above, then anti-CFH antibody (Ox24, Santa Cruz Biotechnologies, sc-53067) was diluted 1:3000 in DMEM 5% FBS and 50 µL was applied to each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above then donkey anti-mouse-HRP antibody was diluted 1:5000 in DMEM 5% FBS and 50 µL was applied to each well and incubated at room temperature for 1 hour.

After 1 hour, the plate was washed as above, then 100 µL TMB reagent was applied to each well and incubated at room temperature in the dark for approximately 15 minutes. Once sufficient blue colour had been obtained, 100 µL 1 M sulphuric acid was added to each well to stop the reaction.

The A450 was then recorded with and data were processed and transferred to Microsoft Excel for analysis.

The FHL1 ELISA results are shown in Figure 4.

Production of AAV2 vectors

The best four (CFI) and five (FHL1) sequences were taken forward to studies using AAV.

HEK293 cells were transfected with the selected codon optimised plasmids along with pRepCap and pHelper as per a typical triple transfection protocol, specifically:

Day 1: HEK293 cells were dissociated and counted using a ViCell. Cells were seeded in a 10 cm dish at 6×10^5 cells per cm^2 in 10 mL DMEM 10% FBS per dish.

Day 2: Confluency was checked and was found to be between 70-80%.

Media was replaced with 10 mL DMEM/Glutamax with 5% FBS.

After 4 hours the cells were transfected with 5 µg plasmid using PEI at a 1:3 DNA:PEI ratio.

Day 3: 15 mM butyrate was added to the 11 mL media in each plate.

Day 5: Supernatant was harvested and centrifuged at 1000 rpm for 10 minutes to remove cell debris.

The supernatant was transferred to a fresh tube and 1/5 vol. AAVanced (AAV110A-1, Cambridge Bioscience) reagent was added (2.75 mL in 11 mL).

The mixture was then stored at 4°C.

Day 8: The supernatant/AAVanced mixture was centrifuged at 1000 rpm for 30 minutes at 4°C.

The supernatant was discarded and the pellet resuspended in 500 µL PBS. This was then transferred to a 1.5 mL tube and centrifuged for 3 minutes at 1500 g.

The supernatant was discarded and the remaining pellet resuspended in 1/100 original volume (i.e. 100 µL per 11 mL supernatant).

The vector was stored at -80°C.

Transduction of ARPE19 cells

ARPE19 cells were transduced with the vectors comprising the codon optimised transgenes.

Day 1: ARPE19 cells were dissociated and counted a ViCell then seeded at 1×10^5 cells per well in 200 µL DMEM/Glutamax + 10% FBS.

Day 2: Vector was added to the cells.

Day 3: Media was replaced with serum-free media.

Day 4: The supernatant was harvested, centrifuged at 14000 rpm for 10 minutes at 4°C, then transferred to a fresh tube.

Total protein concentration was assessed by Bradford Assay.

CFI ELISA

Supernatants from the transduction were analysed by ELISA for CFI according to the protocol above.

The CFI ELISA results are shown in Figure 5.

FHL-1 ELISA

Supernatants from the transduction were analysed by ELISA for FHL1 according to the protocol above.

The FHL1 ELISA results are shown in Figure 6.

CONCLUSIONS

RC136 (CFI; SEQ ID NO: 10) and RC146 (FHL-1; SEQ ID NO: 12) each give higher expression of the transgene than the wild type sequences and other codon-optimised sequences tested.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the disclosed agents, compositions, uses and methods of the invention will be apparent to the skilled person without departing from the scope and spirit of the invention. Although the invention has been disclosed in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the disclosed modes for carrying out the invention, which are obvious to the skilled person are intended to be within the scope of the following claims.

CLAIMS

1. An isolated polynucleotide comprising a nucleotide sequence encoding Complement Factor I (CFI), wherein the nucleotide sequence has at least 85% sequence identity to SEQ ID NO: 10.

2. The isolated polynucleotide of claim 1, wherein the nucleotide sequence is SEQ ID NO: 10.

3. An isolated polynucleotide comprising a nucleotide sequence encoding Complement Factor H-like Protein 1 (FHL1), wherein the nucleotide sequence has at least 75% sequence identity to SEQ ID NO: 12.

4. The isolated polynucleotide of claim 3, wherein the nucleotide sequence is SEQ ID NO: 12.

5. A vector comprising the polynucleotide of any preceding claim.

6. The vector of claim 5, wherein the vector is an adeno-associated viral (AAV) vector.

7. The vector of claim 5 or 6, wherein the vector is in the form of a viral vector particle.

8. The vector of claim 7, wherein the AAV vector particle comprises an AAV2 or AAV8 genome, and AAV2 or AAV8 capsid proteins.

9. The vector of any one of claims 5-8, wherein the nucleotide sequence encoding the CFI or FHL1 is operably linked to a CMV promoter; a WPRE regulatory element; and/or a poly-A signal.

10. A cell comprising the polynucleotide of any one of claims 1-4.

11. A cell transduced with the vector of any one of claims 5-9.

12. A pharmaceutical composition comprising the polynucleotide, vector or cell of any preceding claim in combination with a pharmaceutically acceptable carrier, diluent or excipient.

13. The polynucleotide, vector or cell of any one of claims 1-11 for use in therapy.

14. The polynucleotide, vector or cell of any one of claims 1-11 for use in treating or preventing a complement-mediated disorder of the eye.

15. The polynucleotide, vector or cell for use according to claim 14, wherein the disorder is age-related macular degeneration (AMD) or diabetic retinopathy, preferably AMD.

16. The polynucleotide, vector or cell for use according to claim 15, wherein the AMD is dry AMD.

17. The polynucleotide, vector or cell for use according to any one of claims 13-16, wherein the formation of geographic atrophy is prevented or reduced, and/or the amount of geographic atrophy is reduced.

18. The polynucleotide, vector or cell for use according to any one of claims 13-17, wherein the progression of geographic atrophy is slowed.

19. The polynucleotide, vector or cell for use according to any one of claims 13-18, wherein there is at least a 10% reduction in the increase in geographic atrophy area over the 12 months following administration to a treated eye of a subject, relative to an untreated eye over the same period.

20. The polynucleotide, vector or cell for use according to any one of claims 13-19, wherein administration of the polynucleotide, vector or cell increases the level of C3b-inactivating and iC3b-degradation activity in a subject, or in an eye, such as in the retinal pigment epithelium (RPE), of a subject, optionally to a level that exceeds a normal level in a subject, or eye or RPE thereof.

21. The polynucleotide, vector or cell for use according to any one of claims 13-20, wherein the polynucleotide, vector or cell is administered intraocularly.

22. The polynucleotide, vector or cell for use according to any one of claims 13-21, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal, direct retinal, suprachoroidal or intravitreal injection.

23. The polynucleotide, vector or cell for use according to any one of claims 13-22, wherein the polynucleotide, vector or cell is administered to the eye of a subject by subretinal injection.

24. A method of treating or preventing a complement-mediated disorder of the eye comprising administering the polynucleotide, vector or cell of any one of claims 1-11 to a subject in need thereof.

25. A method of providing Complement Factor I (CFI) and/or Complement Factor H-like Protein 1 (FHL1) to a subject, comprising delivering the polynucleotide, vector or cell of any one of claims 1-11 to the eye of the subject.