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(54) **Title:** HYBRID RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE BETWEEN AAV9 AND AAVrh74 WITH REDUCED LIVER TROPISM

(57) **Abstract:** The invention relates to a recombinant adeno-associated virus (AAV) capsid protein, which is a hybrid between AAV serotype 9 (AAV9) and AAV serotype 74 (AAVrh74) capsid proteins, wherein said recombinant hybrid AAV capsid protein has a reduced liver tropism compared to the parent AAV9 and AAVrh74 capsid proteins. The invention relates also to the derived hybrid AAV serotype vector particles packaging a gene of interest and their use in gene therapy, in particular for treating neuromuscular genetic diseases.

HYBRID RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE BETWEEN AAV9 AND AAVrh74 WITH REDUCED LIVER TROPISM

FIELD OF THE INVENTION

The present invention relates to a recombinant adeno-associated virus (AAV) capsid, which is a hybrid between AAV serotype 9 (AAV9) and AAV serotype rh74 (AAVrh74) capsid proteins having a reduced liver tropism compared to the parent AAV9 and AAVrh74 capsid proteins. The invention relates also to the derived hybrid AAV serotype vector particles packaging a gene of interest, and their use in gene therapy, in particular for treating neuromuscular genetic diseases.

BACKGROUND OF THE INVENTION

Recombinant Adeno-Associated Virus (rAAV) vectors are widely used for *in vivo* gene transfer. rAAV vectors are non-enveloped vectors composed of a capsid of 20 nm of diameter and a single strand DNA of 4.7 kb. The genome carries two genes, rep and cap, flanked by two palindromic regions named Inverted terminal Repeats (ITR). The cap gene codes for three structural proteins VP1, VP2 and VP3 that compose the AAV capsid. VP1, VP2 and VP3 share the same C-terminal end which is all of VP3. Using AAV2 has a reference, VP1 has a 735 amino acid sequence (GenBank YP_680426); VP2 (598 amino acids) starts at the Threonine 138 (T138) and VP3 (533 amino acids) starts at the methionine 203 (M203). AAV serotypes are defined by their capsid. Different serotypes exist, each of them displaying its own tissue targeting specificity. Therefore, the choice of using a serotype depends on the tissue to transduce. Skeletal muscle and liver tissues are infected and transduced efficiently by different serotypes of AAV vectors such as AAV8, AAV9 and AAV-rh74.

Chimeric or hybrid AAV serotypes have been generated by exchanging fragments of capsid sequences between capsids of different naturally occurring AAV serotypes, in order to

increase AAV transduction efficiency or increase AAV tropism to a cell or tissue type of interest.

Hybrid AAV capsids were generated by combining structural domains of capsids of AAV8 and AAV serotypes isolated from primate brain. The resulting AAV hybrid serotypes can transduce retinal tissue in human and mice with no increase in efficiency compared to AAV2 and AAV5 vectors (Charbel Issa et al., PLOS ONE, 2013, 8, e60361). However, one of the hybrid AAV serotype shows improved transduction efficiency for fat tissue compared to AAV1, AAV8 and AAV9 (Liu et al., Molecular Therapy, 2014, 1, 8, doi:10.1038/mtm).

WO 2015/191508 discloses recombinant hybrid AAV capsids generated by exchanging variable regions of AAV capsids from various species (human, primate, avian, snake, bovine.), in particular AAV capsids with central nervous system tropism to generate CNS specific chimeric capsids.

WO 2017/096164 discloses recombinant hybrid AAV capsids between AAV1, AAV2, AAV3b, AAV6 and AAV8 serotypes exhibiting enhanced human skeletal muscle tropism.

However, all naturally occurring AAV serotypes and variants tested to date have a propensity to accumulate within the liver. This causes problems, in particular when the AAV vector is administered by the systemic route. Firstly, a transgene aimed to be expressed in muscle may have toxic effects on the liver. Secondly, AAV vector entry in liver reduces the amount of vector available for skeletal muscles. Consequently, higher doses of AAV vectors are required. This increases liver toxicity and cost of vector production.

Tissue-specific promoters and microRNA-based gene regulation strategies have been used to segregate gene expression patterns among different tissue types. However, such regulatory strategies do not preclude sequestration of AAV vector genomes in off-target organs such as the liver after systemic administration.

Attenuation of heparin binding by mutating the basic residues R585 or R588 of the capsid protein was shown to abolish heparin sulfate binding and reduce the liver tropism of AAV2-derived vectors (Asokan *et al.*, Nat. Biotechnol., 2010, 28, 79-82). However, this strategy can only work for serotypes like AAV2 and AAV6 whose liver tropism is determined by basic residues binding to heparin.

Therefore, there is a need for new AAV vectors, having a liver tropism which is much lower than their muscle tropism. In addition, new vectors that could infect muscles efficiently but could not infect the liver nor the brain would be even more desirable.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a recombinant adeno-associated virus (AAV) capsid protein, which is a hybrid between AAV serotype 9 (AAV9) and AAV serotype 74 (AAVrh74) capsid proteins, wherein said recombinant hybrid AAV capsid protein has a reduced liver tropism compared to the parent AAV9 and AAVrh74 capsid proteins, and wherein said recombinant hybrid AAV capsid protein comprises a sequence selected from the group consisting of the sequences SEQ ID NO: 3 and SEQ ID NO: 4 and the sequences having at least 85% identity with said sequences, preferably SEQ ID NO:3.

In a second aspect, the present invention provides a recombinant chimeric AAV capsid protein, which is selected from the group consisting of:

- a chimeric VP1 protein comprising: (i) a VP1-specific N-terminal region having a sequence from natural or artificial AAV serotype other than AAV9 and AAVrh74, (ii) a VP2-specific N-terminal region having a sequence from AAV9, AAVrh74 or natural or artificial AAV serotype other than AAV9 and AAVrh74, and (iii) a VP3 C-terminal region having the sequence of a hybrid VP3 protein according to the first aspect, and

- a chimeric VP2 protein comprising: (i) a VP2-specific N-terminal region having a sequence from natural or artificial AAV serotype other than AAV9 and AAVrh74, and (ii) a VP3 C-terminal region having the sequence of a hybrid VP3 protein according to the first aspect.

In a third aspect, the present invention provides a polynucleotide encoding the recombinant hybrid AAV capsid protein according to the first aspect or the recombinant chimeric AAV capsid protein according to the second aspect, in expressible form, and eventually further encoding AAV Replicase protein in expressible form.

In a fourth aspect, the present invention provides a recombinant plasmid comprising the polynucleotide of the third aspect.

In a fifth aspect, the present invention provides an AAV vector particle packaging a gene of interest, which comprises the hybrid recombinant AAV capsid protein according to the first aspect, and/or the recombinant chimeric AAV capsid protein according to the second aspect, and eventually also at least one AAV capsid protein from natural or artificial AAV serotype other than AAV9 and AAVrh74.

In a sixth aspect, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of AAV vector particles according to the fifth aspect.

In a seventh aspect, the present invention provides a method of gene therapy comprising administering to a patient a therapeutically effective amount of the pharmaceutical composition according to the sixth aspect.

In an eighth aspect, the present invention provides use of AAV vector particles according to the fifth aspect, or the pharmaceutical composition according to claim 13, in the manufacture of a medicament for providing gene therapy to the sixth aspect.

The inventors have generated new hybrid AAV serotypes using a combination of two serotypes that infect efficiently the muscle and liver tissues, AAV9 and AAV-rh74. Two new hybrid AAV serotypes were generated using the swapping of a variable region of the cap gene between the AAV9 and AAVrh74 serotypes (*Error! Reference source not found.***A and 1B**). Surprisingly, the liver tropism of the parent AAV9 and AAVrh74 was lost in the hybrid AAV serotype **(Figure 4C and 4D)**. At the same time, the hybrid AAV serotype exhibited high titer AAV vector production and high level gene transduction efficiencies in skeletal and cardiac muscle tissues.

The new hybrid AAV serotypes are useful in gene therapy of neuromuscular disorders, including genetic diseases, autoimmune diseases, neurodegenerative diseases and cancer.

Therefore, the invention encompasses a hybrid recombinant AAV capsid between AAV9 and AAVrh74 capsids with reduced liver tropism, AAV vector particles comprising the hybrid recombinant AAV capsid, compositions comprising the hybrid AAV serotype vector particles, and methods of making and using said hybrid AAV serotype vector particles and compositions, in particular in gene therapy.

DETAILED DESCRIPTION OF THE INVENTION

Recombinant hybrid AAV capsid protein

One aspect of the invention relates to a recombinant adeno-associated virus (AAV) capsid protein, which is a hybrid between AAV serotype 9 (AAV9) and AAV serotype 74 (AAVrh74) capsid proteins, wherein said recombinant hybrid AAV capsid protein has reduced liver tropism compared to its parent AAV9 and AAVrh74 capsid proteins.

As used herein, the term "tropism" refers to the specificity of an AAV capsid protein present in an AAV viral particle, for infecting a particular type of cell or tissue.

The tropism of an AAV capsid for a particular type of cell or tissue may be determined by measuring the ability of AAV vector particles comprising the hybrid AAV capsid protein to infect or to transduce a particular type of cell or tissue, using standard assays that are well-known in the art such as those disclosed in the examples of the present application.

As used herein, the term "liver tropism" or "hepatic tropism" refers to the tropism for liver or hepatic tissue and cells, including hepatocytes.

According to the invention, the liver tropism of the hybrid AAV capsid protein is reduced by at least 20 %, 30%, 40%, 50% or more; preferably at least 50%, 60% 70%, 80%, 90% or 99% compared to the liver tropism of the parent AAV9 or AAVrh74 capsid protein.

According to the invention, the hybrid AAV capsid protein has tropism for muscle cells and tissues.

Muscle tissues include in particular cardiac and skeletal muscle tissues.

As used herein, the term "muscle cells" refers to myocytes, myotubes, myoblasts, and/or satellite cells.

In some embodiments, the muscle tropism of the hybrid AAV capsid protein is similar to that of its parent AAV9 and/or AAVrh74 capsid proteins. Preferably, the muscle tropism of the hybrid AAV capsid protein is equivalent to at least 50%, 60%, 70%, 80%, 90%, 99% or more of that of the parent AAV9 and/or AAVrh74 capsid protein.

In some embodiments, the hybrid AAV capsid protein is a hybrid VP1, VP2 or VP3 protein.

In some embodiments, the hybrid AAV capsid protein has tropism for at least skeletal muscle tissue. In some preferred embodiments, the hybrid AAV capsid protein has tropism for both skeletal and cardiac muscle tissues. An example of this type of hybrid is the hybrid AAV capsid of SEQ ID NO: 3 (named Hybrid Cap9-rh74 in the examples). This type of hybrid AAV capsid is useful for the treatment of cardiac and skeletal muscle disorders.

The hybrid AAV capsid protein according to the invention may be derived from any AAV9 and AAVrh74 capsid protein sequences; such sequences are well-known in the art and available in public sequence data base. For example, AAV9 capsid protein corresponds to GenBank accession numbers: AY530579.1; SEQ ID NO: 123 of WO 2005/033321; SEQ ID

NO: 1 of WO 2012/112832; AAV9 capsid variants in which one or more of the native residues at positions 271 (D), 446(Y), and 470 (N) are replaced with another amino acid, preferably alanine as disclosed in WO 2012/112832; AAV9 capsid variants at one or more of positions K143R, T251A, S499A, S669A and S490A as disclosed in US 2014/0162319. AAVrh74 capsid protein corresponds to SEQ ID NO: 1 of WO 2015/013313; SEQ ID NO: 6 of WO 2006/110689; SEQ ID NO: 1 of WO 2013/123503; SEQ ID NO: 4 of WO 2013/158879; and K137R, K333R, K550R, K552R, K569R, K691R, K695R, K709R variants and combination thereof.

In some embodiments, the hybrid AAV capsid protein according to the invention is derived from the AAV9 capsid protein of SEQ ID NO: 1 (GenBank AY530579.1) and the AAVrh74 protein of SEQ ID NO: 2.

In some embodiments, the hybrid AAV capsid protein according to the invention results from the replacement of a variable region in the AAV9 or AAVrh74 capsid sequence with the corresponding variable region of the other AAV serotype capsid sequence,

wherein the variable region of AAV9 capsid corresponds to the sequence situated from any one of positions 331 to 493 to any one of positions 556 to 736 in AAV9 capsid of SEQ ID NO: 1 (reference sequence), or a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 consecutive amino acids of the sequence situated from positions 493 to 556 in AAV9 capsid of SEQ ID NO: 1, and

the variable region of AAVrh74 capsid corresponds to the sequence situated from any one of positions 332 to 495 to any one of positions 558 to 738 in AAVrh74 capsid of SEQ ID NO: 2 (reference sequence), or a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 consecutive amino acids of the sequence situated from positions 495 to 558 in AAVrh74 capsid of SEQ ID NO: 2.

The invention encompasses hybrid AAV capsid proteins derived from any AAV9 and AAVrh74 capsid protein sequences by replacement of a variable region region in the AAV9 or AAVrh74 capsid sequence with the corresponding variable region of the other AAV serotype capsid sequence, as defined above. According to the invention, the variable region is defined using AAV9 capsid of SEQ ID NO: 1 and AAVrh74 capsid of SEQ ID NO: 2 as reference. After sequence alignment of any other AAV9 capsid sequence with SEQ ID NO: 1 or any of other AAVrh74 capsid sequence with SEQ ID NO: 2, using standard protein

sequence alignment programs that are well-known in the art, such as for example BLAST, FASTA, CLUSTALW, and the like, a person skilled in the art can easily obtained the corresponding positions of the variable region in other AAV9 or AAVrh74 capsid sequences.

In some preferred embodiments, the hybrid AAV capsid protein according to the invention results from the replacement of the variable region corresponding to that situated from positions 449 to 609 in the AAV9 capsid sequence of SEQ ID NO: 1 or from positions 450 to 611 in the AAVrh74 capsid sequence of SEQ ID NO: 2 with the corresponding variable region of the other serotype.

In some embodiments, said hybrid AAV capsid protein comprises a sequence selected from the group consisting of the sequences SEQ ID NO: 3 and SEQ ID NO: 4, the sequences having at least 85%, 90%, 95%, 97%, 98% or 99% identity with said sequences, and the fragment thereof corresponding to VP2 or VP3 capsid protein. VP2 corresponds to the amino acid sequence from T138 to the end of SEQ ID NO: 3 or 4. VP3 corresponds to the amino acid sequence from M203 to the end of SEQ ID NO: 3 or from M204 to the end of SEQ ID NO: 4.

SEQ ID NO: 3 is derived from AAV9 capsid protein of SEQ ID NO: 1 by replacement of AAV9 variable region (positions 449 to 609 of SEQ ID NO: 1) with the variable region of AAVrh74 capsid protein (positions 450 to 611 of SEQ ID NO: 2); the corresponding hybrid is named Hybrid Cap9-rh74 in the examples. VP2 corresponds to the amino acid sequence from T138 to the end of SEQ ID NO: 3. VP3 corresponds to the amino acid sequence from M203 to the end of SEQ ID NO: 3.

SEQ ID NO: 4 is derived from AAVrh74 capsid protein of SEQ ID NO: 2 by replacement of rh74 variable region (positions 450 to 611 of SEQ ID NO: 2) with the variable region of AAV9 capsid protein (positions 449 to 609 of SEQ ID NO: 1); the corresponding hybrid is named Hybrid Caprh74-9 in the examples. VP2 corresponds to the amino acid sequence from T138 to the end of SEQ ID NO: 4. VP3 corresponds to the amino acid sequence from M204 to the end of SEQ ID NO: 4.

In some preferred embodiments, the hybrid AAV capsid protein according to the invention is derived from AAV9 capsid protein by replacement of a variable region of AAV9 capsid sequence with the corresponding variable region of AAVrh74 capsid sequence as defined above, preferably the hybrid AAV capsid protein comprises the replacement of the variable region corresponding to that situated from positions 449 to 609 in AAV9 capsid of SEQ ID NO: 1 with the variable region corresponding to that situated from positions 450 to 611 in AAVrh74 capsid of SEQ ID NO: 2. Preferably, said hybrid AAV capsid protein comprises a sequence selected from the group consisting of the sequence of SEQ ID NO: 3 and the sequences having at least 85%, 90%, 95%, 97%, 98% or 99% identity with said sequence; more preferably which comprises the sequence of SEQ ID NO: 3.

The term "identity" refers to the sequence similarity between two polypeptide molecules or between two nucleic acid molecules. When a position in both compared sequences is occupied by the same base or same amino acid residue, then the respective molecules are identical at that position. The percentage of identity between two sequences corresponds to the number of matching positions shared by the two sequences divided by the number of positions compared and multiplied by 100. Generally, a comparison is made when two sequences are aligned to give maximum identity. The identity may be calculated by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA or CLUSTALW.

In some embodiments, the hybrid AAV capsid protein of the invention generates high yields of recombinant AAV vector particles. Preferably, the titer of the hybrid capsid recombinant AAV vector is equal or superior to 10^{11} viral genomes per mL (vg /mL). High yields of recombinant AAV vector particles are useful for gene therapy applications.

The invention encompasses also AAV VP1 and VP2 chimeric capsid proteins derived from the AAV9/rh74 hybrid VP3 capsid protein according to the invention, wherein the VP1-specific N-terminal region and/or VP2-specific N-terminal region are from a natural or artificial AAV serotype other than AAV9 and AAVrh74.

In some embodiments, the AAV VP1 chimeric capsid protein comprises:

(i) a VP1-specific N-terminal region having a sequence from natural or artificial AAV serotype other than AAV9 and AAVrh74,

(ii) a VP2-specific N-terminal region having a sequence from AAV9, AAVrh74 or natural or artificial AAV serotype other than AAV9 and AAVrh74, and

(iii) a VP3 C-terminal region having the sequence of a hybrid VP3 protein according to the invention.

In some embodiments, the AAV VP2 chimeric capsid proteins comprises

(i) a VP2-specific N-terminal region having a sequence from natural or artificial AAV serotype other than AAV9 and AAVrh74, and

(ii) a VP3 C-terminal region having the sequence of a hybrid VP3 protein according to the invention.

Polynucleotide, vector, and use for AAV vector production

Another aspect of the invention is a polynucleotide encoding the recombinant hybrid AAV capsid protein in expressible form. The polynucleotide may be DNA, RNA or a synthetic or semi-synthetic nucleic acid.

In some embodiments, the polynucleotide is a AAV9/rh74 hybrid cap gene encoding hybrid VP1, VP2 and VP3 capsid proteins according to the invention. In some preferred embodiments, the polynucleotide comprises the sequence SEQ ID NO: 5 (encoding the hybrid AAV capsid protein of SEQ ID NO: 3) or the sequence SEQ ID NO: 7 (encoding the hybrid AAV capsid protein of SEQ ID NO: 4).

In some other embodiments, the polynucleotide is a chimeric cap gene which codes for a AAV9/rh74 hybrid VP3 capsid protein according to the invention and a chimeric VP1 capsid protein, and maybe also a chimeric VP2 capsid protein wherein the VP1-specific N-terminal region, and maybe also the VP2-specific N-terminal region, are from a natural or artificial AAV serotype other than AAV9 and AAVrh74. Such chimeric cap gene may be generated by any suitable technique, using the coding sequence for an AAV9/rh74 hybrid VP3 capsid protein according to the invention in combination with heterologous sequences which may be obtained from different selected AAV serotypes, non-contiguous portions of the same AAV serotypes, from a non-viral AAV source or from a non-viral source.

In some embodiments, the polynucleotide further encodes AAV Replicase (Rep) protein in expressible form, preferably Rep from AAV2.

The polynucleotide is advantageously inserted into a recombinant vector, which includes, in a non-limiting manner, linear or circular DNA or RNA molecules consisting of chromosomal, non-chromosomal, synthetic or semi-synthetic nucleic acids, such as in particular viral vectors, plasmid or RNA vectors.

Numerous vectors into which a nucleic acid molecule of interest can be inserted in order to introduce it into and maintain it in a eukaryotic host cell are known *per se*; the choice of an appropriate vector depends on the use envisioned for this vector (for example, replication of the sequence of interest, expression of this sequence, maintaining of this sequence in extrachromosomal form, or else integration into the chromosomal material of the host), and also on the nature of the host cell.

In some embodiments, the vector is a plasmid.

The recombinant vector for use in the present invention is an expression vector comprising appropriate means for expression of the hybrid AAV capsid protein, and maybe also AAV Rep protein. Usually, each coding sequence (hybrid AAV Cap and AAV Rep) is inserted in a separate expression cassette either in the same vector or separately. Each expression cassette comprises the coding sequence (open reading frame or ORF) functionally linked to the regulatory sequences which allow the expression of the corresponding protein in AAV producer cells, such as in particular promoter, promoter/enhancer, initiation codon (ATG), stop codon, transcription termination signal. Alternatively, the hybrid AAV Cap and the AAV Rep proteins may be expressed from a unique expression cassette using an Internal Ribosome Entry Site (IRES) inserted between the two coding sequences or a viral 2A peptide. In addition, the codon sequences encoding the hybrid AAV Cap, and AAV Rep if present, are advantageously optimized for expression in AAV producer cells, in particular human producer cells.

The vector, preferably a recombinant plasmid, is useful for producing hybrid AAV vectors comprising the hybrid AAV capsid protein of the invention, using standard AAV production methods that are well-known in the art (Review in Aponte-Ubillus *et al.*, Applied Microbiology and Biotechnology, 2018, 102: 1045-1054).

Following co-transfection, the cells are incubated for a time sufficient to allow the production of AAV vector particles, the cells are then harvested, lysed, and AAV vector particles are purified by standard purification methods such as for example Cesium Chloride density gradient ultracentrifugation.

AAV particle, pharmaceutical composition and therapeutic uses

Another aspect of the invention is an AAV particle comprising the hybrid recombinant AAV capsid protein of the invention. The AAV particle may comprise hybrid VP1, VP2 and VP3 capsid proteins encoded by a hybrid cap gene according to the invention. Alternatively or additionally, the AAV particle may comprise chimeric VP1 and VP2 capsid proteins and a hybrid VP3 protein encoded by a chimeric cap gene according to the invention.

In some embodiments, the AAV particle is a mosaic AAV particle further comprising another AAV capsid protein from a natural or artificial AAV serotype other than AAV9 and AAVrh74 serotype, wherein the mosaic AAV particle has a reduced liver tropism compared to AAV9 and AAVrh74 serotypes. An artificial AAV serotype may be with no limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a humanized AAV capsid. Such an artificial capsid may be generated by any suitable technique, using a selected AAV sequence (e.g. a fragment of a VP1 capsid protein) in combination with heterologous sequences which may be obtained from a different selected AAV serotype, non-contiguous portions of the same AAV serotype, from a non-viral AAV source or from a non-viral source.

Preferably, the AAV particle is an AAV vector particle. The genome of the AAV vector may either be a single-stranded or self-complementary double-stranded genome (McCarty et al, Gene Therapy, 2003, Dec., 10(26), 2112-2118). Self-complementary vectors are generated by deleting the terminal resolution site (trs) from one of the AAV terminal repeats. These modified vectors, whose replicating genome is half the length of the wild-type AAV genome have the tendency to package DNA dimers. The AAV genome is flanked by ITRs. In particular embodiments, the AAV vector is a pseudotyped vector, *i.e.* its genome and capsid are derived from AAVs of different serotypes. In some preferred embodiments, the genome of the pseudotyped vector is derived from AAV2.

In some preferred embodiments, the AAV vector particle is packaging a gene of interest.

The AAV particle may be obtained using the method of producing recombinant AAV vector particles of the invention.

By “gene of interest”, it is meant a gene useful for a particular application, such as with no limitation, diagnosis, reporting, modifying, therapy and genome editing.

For example, the gene of interest may be a therapeutic gene, a reporter gene or a genome-editing enzyme.

By "gene of interest for therapy", "gene of therapeutic interest", or "heterologous gene of interest", it is meant a therapeutic gene or a gene encoding a therapeutic protein, peptide or RNA.

The gene of interest is any nucleic acid sequence capable of modifying a target gene or target cellular pathway, in particular in muscle cells. For example, the gene may modify the expression, sequence or regulation of the target gene or cellular pathway. In some embodiments, the gene of interest is a functional version of a gene or a fragment thereof. The functional version of said gene includes the wild-type gene, a variant gene such as variants belonging to the same family and others, or a truncated version, which preserves the functionality of the encoded protein at least partially. A functional version of a gene is useful for replacement or additive gene therapy to replace a gene, which is deficient or non-functional in a patient. In other embodiments, the gene of interest is a gene which inactivates a dominant allele causing an autosomal dominant genetic disease. A fragment of a gene is useful as recombination template for use in combination with a genome editing enzyme.

Alternatively, the gene of interest may encode a protein of interest for a particular application, (for example an antibody or antibody fragment, a genome-editing enzyme) or a RNA. In some embodiments, the protein is a therapeutic protein including a therapeutic antibody or antibody fragment, or a genome-editing enzyme. In some embodiments, the RNA is a therapeutic RNA. The gene of interest is a functional gene able to produce the encoded protein, peptide or RNA in the target cells of the disease, in particular muscle cells. The AAV viral vector comprises the gene of interest in a form expressible in muscle cells, including cardiac and skeletal muscle cells. In particular, the gene of interest is operatively linked to a ubiquitous, tissue-specific or inducible promoter which is functional in muscle cells. The gene of interest may be inserted in an expression cassette further comprising polyA sequences.

The RNA is advantageously complementary to a target DNA or RNA sequence or binds to a target protein. For example, the RNA is an interfering RNA such as a shRNA, a microRNA, a guide RNA (gRNA) for use in combination with a Cas enzyme or similar enzyme for genome editing, an antisense RNA capable of exon skipping such as a modified small

nuclear RNA (snRNA) or a long non-coding RNA. The interfering RNA or microRNA may be used to regulate the expression of a target gene involved in muscle disease. The guide RNA in complex with a Cas enzyme or similar enzyme for genome editing may be used to modify the sequence of a target gene, in particular to correct the sequence of a mutated/deficient gene or to modify the expression of a target gene involved in a disease, in particular a neuromuscular disease. The antisense RNA capable of exon skipping is used in particular to correct a reading frame and restore expression of a deficient gene having a disrupted reading frame. In some embodiments, the RNA is a therapeutic RNA.

The genome-editing enzyme according to the invention is any enzyme or enzyme complex capable of modifying a target gene or target cellular pathway, in particular in muscle cells. For example, the genome-editing enzyme may modify the expression, sequence or regulation of the target gene or cellular pathway.The genome-editing enzyme is advantageously an engineered nuclease, such as with no limitations, a meganuclease, zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALENs), Cas enzyme from clustered regularly interspaced palindromic repeats (CRISPR)-Cas system and similar enzymes. The genome-editing enzyme, in particular an engineered nuclease such as Cas enzyme and similar enzymes, may be a functional nuclease which generates a double-strand break (DSB) in the target genomic locus and is used for site-specific genome editing applications, including with no limitations: gene correction, gene replacement, gene knock-in, gene knock-out, mutagenesis, chromosome translocation, chromosome deletion, and the like. For site-specific genome editing applications, the genome-editing enzyme, in particular an engineered nuclease such as Cas enzyme and similar enzymes may be used in combination with a homologous recombination (HR) matrix or template (also named DNA donor template) which modifies the target genomic locus by double-strand break (DSB)-induced homologous recombination. In particular, the HR template may introduce a transgene of interest into the target genomic locus or repair a mutation in the target genomic locus, preferably in an abnormal or deficient gene causing a neuromuscular disease. Alternatively, the genome-editing enzyme, such as Cas enzyme and similar enzymes may be engineered to become nuclease-deficient and used as DNA-binding protein for various genome engineering applications such as with no limitation: transcriptional activation, transcriptional repression, epigenome modification, genome imaging, DNA or RNA pull-down and the like.

Another aspect of the invention is a pharmaceutical composition comprising a therapeutically effective amount of AAV particles comprising the hybrid recombinant AAV capsid protein of the invention, preferably AAV vector particles packaging a therapeutic gene of interest.

In some embodiments of the invention, the pharmaceutical composition of the invention is for use as a medicament, in particular in gene therapy. The invention encompasses the use of the pharmaceutical composition of the invention as a medicament, in particular for the treatment of a disease by gene therapy.

Gene therapy can be performed by gene transfer, gene editing, exon skipping, RNA-interference, trans-splicing or any other genetic modification of any coding or regulatory sequences in the cell, including those included in the nucleus, mitochondria or as commensal nucleic acid such as with no limitation viral sequences contained in cells.

The two main types of gene therapy are the following:

- a therapy aiming to provide a functional replacement gene for a deficient/abnormal gene: this is replacement or additive gene therapy;
- a therapy aiming at gene or genome editing: in such a case, the purpose is to provide to a cell the necessary tools to correct the sequence or modify the expression or regulation of a deficient/abnormal gene so that a functional gene is expressed or an abnormal gene is suppressed (inactivated): this is gene editing therapy.

In additive gene therapy, the gene of interest may be a functional version of a gene, which is deficient or mutated in a patient, as is the case for example in a genetic disease. In such a case, the gene of interest will restore the expression of a functional gene.

Gene or genome editing uses one or more gene(s) of interest, such as:

(i) a gene encoding a therapeutic RNA as defined above such as an interfering RNA like a shRNA or a microRNA, a guide RNA (gRNA) for use in combination with a Cas enzyme or similar enzyme, or an antisense RNA capable of exon skipping such as a modified small nuclear RNA (snRNA); and

(ii) a gene encoding a genome-editing enzyme as defined above such as an engineered nuclease like a meganuclease, zinc finger nuclease (ZFN), transcription activator-like

effector-based nuclease (TALENs), Cas enzyme or similar enzymes; or a combination of such genes, and maybe also a fragment of a functional version of a gene for use as recombination template, as defined above.

Gene therapy is used for treating various diseases, including with no limitations, genetic diseases, in particular neuromuscular genetic disorders, cancer, neurodegenerative diseases and auto-immune diseases.

In some embodiments, gene therapy is used for treating diseases affecting muscle tissues, in particular skeletal muscle tissue and/or cardiac tissue, such as with no-limitations: neuromuscular genetic disorders, cardiomyopathies, rhabdomyosarcomas, Polymyositis, Dermatomyositis, juvenile polymyositis and others.

Examples of mutated genes in neuromuscular genetic disorders that can be targeted by gene therapy using the pharmaceutical composition of the invention are listed in the following tables:

Muscular dystrophies

Gene	Protein
DMD	Dystrophin
EMD	Emerin
FHL1	Four and a half LIM domain 1
LMNA	Lamin A/C
SYNE1	Spectrin repeat containing, nuclear envelope 1 (nesprin 1)
SYNE2	Spectrin repeat containing, nuclear envelope 2 (nesprin 2)
TMEM43	Transmembrane protein 43
TOR1AIP1	Torsin A interacting protein 1
DUX4	Double homeobox 4
SMCHD1	Structural maintenance of chromosomes flexible hinge domain containing 1
PTRF	Polymerase I and transcript release factor
MYOT	Myotilin
CAV3	Caveolin 3
DNAJB6	HSP-40 homologue, subfamily B, number 6
DES	Desmin
TNPO3	Transportin 3
HNRNPDL	Heterogeneous nuclear ribonucleoprotein D-like
CAPN3	Calpain 3
DYSF	Dysferlin

SGCG	Gamma sarcoglycan
SGCA	Alpha sarcoglycan
SGCB	Beta sarcoglycan
SGCD	Delta-sarcoglycan
TCAP	Telethonin
TRIM32	Tripartite motif-containing 32
FKRP	Fukutin-related protein
TTN	Titin
POMT1	Protein-O-mannosyltransferase 1
ANO5	Anoctamin 5
FKTN	Fukutin
POMT2	Protein-O-mannosyltransferase 2
POMGNT1	O-linked mannose beta1,2-N-acetylglucosaminyltransferase
PLEC	Plectin
TRAPPC11	trafficking protein particle complex 11
GMPPB	GDP-mannose pyrophosphorylase B
DAG1	Dystroglycan1
DPM3	Dolichyl-phosphate mannosyltransferase polypeptide 3
ISPD	Isoprenoid synthase domain containing
VCP	Valosin-containing protein
LIMS2	LIM and senescent cell antigen-like domains 2
GAA	Glucosidase alpha, acid

Congenital muscular dystrophies

Gene	**Protein**
LAMA2	Laminin alpha 2 chain of merosin
COL6A1	Alpha 1 type VI collagen
COL6A2	Alpha 2 type VI collagen
COL6A3	Alpha 3 type VI collagen
SEPN1	Selenoprotein N1
FHL1	Four and a half LIM domain 1
ITGA7	Integrin alpha 7 precursor
DNM2	Dynamin 2
TCAP	Telethonin
LMNA	Lamin A/C
FKTN	Fukutin
POMT1	Protein-O-mannosyltransferase 1
POMT2	Protein-O-mannosyltransferase 2
FKRP	Fukutin-related protein
POMGNT1	O-linked mannose beta1,2-N-acetylglucosaminyltransferase

ISPD	Isoprenoid synthase domain containing
POMGNT2	protein O-linked mannose N-acetylglucosaminyltransferase 2
B3GNT1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyl-transferase 1
GMPPB	GDP-mannose pyrophosphorylase B
LARGE	Like-glycosyltransferase
DPM1	Dolichyl-phosphate mannosyltransferase 1, catalytic subunit
DPM2	Dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit
ALG13	UDP-N-acetylglucosami-nyltransferase
B3GALNT2	Beta-1,3-N-acetylgalacto-saminyltransferase 2
TMEM5	Transmembrane protein 5
POMK	Protein-O-mannose kinase
CHKB	Choline kinase beta
ACTA1	Alpha actin, skeletal muscle
TRAPPC11	trafficking protein particle complex 11

Congenital myopathies

Gene	Protein
TPM3	Tropomyosin 3
NEB	Nebulin
ACTA1	Alpha actin, skeletal muscle
TPM2	Tropomyosin 2 (beta)
TNNT1	Slow troponin T
KBTBD13	Kelch repeat and BTB (POZ) domain containing 13
CFL2	Cofilin 2 (muscle)
KLHL40	Kelch-like family member 40
KLHL41	Kelch-like family member 41
LMOD3	Leiomodin 3 (fetal)
SEPN1	Selenoprotein N1
RYR1	Ryanodine receptor 1 (skeletal)
MYH7	Myosin, heavy polypeptide 7, cardiac muscle, beta
MTM1	Myotubularin
DNM2	Dynamin 2
BIN1	Amphiphysin
TTN	Titin
SPEG	SPEG complex locus
MEGF10	Multiple EGF-like-domains 10
MYH2	Myosin, heavy polypeptide 2, skeletal muscle
MYBPC3	Cardiac myosin binding protein-C
CNTN1	Contactin-1

TRIM32	Tripartite motif-containing 32
PTPLA	Protein tyrosine phosphatase-like (3-Hydroxyacyl-CoA dehydratase
CACNA1S	Calcium channel, voltage-dependent, L type, alpha 1S subunit

Distal myopathies

Gene symbol	**protein**
DYSF	Dysferlin
TTN	Titin
GNE	UDP-N-acetylglucosamine-2- epimerase/N-acetylmannosamine kinase
MYH7	Myosin, heavy polypeptide 7, cardiac muscle, beta
MATR3	Matrin 3
TIA1	Cytotoxic granuleassociated RNA binding protein
MYOT	Myotilin
NEB	Nebulin
CAV3	Caveolin 3
LDB3	LIM domain binding 3
ANO5	Anoctamin 5
DNM2	Dynamin 2
KLHL9	Kelch-like homologue 9
FLNC	Filamin C, gamma (actin-binding protein - 280)
VCP	Valosin-containing protein

Other myopathies

Gene symbol	**protein**
ISCU	Iron-sulfur cluster scaffold homolog (E. coli)
MSTN	Myostatin
FHL1	Four and a half LIM domain 1
BAG3	BCL2-associated athanogene 3
ACVR1	Activin A receptor, type II-like kinase 2
MYOT	Myotilin
FLNC	Filamin C, gamma (actin-binding protein - 280)
LDB3	LIM domain binding 3
LAMP2	Lysosomal-associated membrane protein 2 precursor
VCP	Valosin-containing protein
CAV3	Caveolin 3
SEPN1	Selenoprotein N1
CRYAB	Crystallin, alpha B
DES	Desmin

VMA21	VMA21 Vacuolar H+-ATPase Homolog (S. Cerevisiae)
PLEC	plectin
PABPN1	Poly(A) binding protein, nuclear 1
TTN	Titin
RYR1	Ryanodine receptor 1 (skeletal)
CLN3	Ceroid-lipofuscinosis, neuronal 3 (=battenin)
TRIM54	
TRIM63	Tripartite motif containing 63, E3 ubiquitin protein ligase

Myotonic syndromes

Gene	**protein**
DMPK	Myotonic dystrophy protein kinase
CNPB	Cellular nucleic acid-binding protein
CLCN1	Chloride channel 1, skeletal muscle (Thomsen disease, autosomal dominant)
CAV3	Caveolin 3
HSPG2	Perlecan
ATP2A1	ATPase, Ca++ transporting, fast twitch 1

Ion Channel muscle diseases

Gene	**protein**
CLCN1	Chloride channel 1, skeletal muscle (Thomsen disease, autosomal dominant)
SCN4A	Sodium channel, voltage-gated, type IV, alpha
SCN5A	Voltage-gated sodium channel type V alpha
CACNA1S	Calcium channel, voltage-dependent, L type, alpha 1S subunit
CACNA1A	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
KCNE3	Potassium voltage-gated channel, Isk-related family, member 3
KCNA1	Potassium voltage-gated channel, shaker-related subfamily, member 1
KCNJ18	Kir2.6 (inwardly rectifying potassium channel 2.6)
KCNJ2	Potassium inwardly-rectifying channel J2
KCNH2	Voltage-gated potassium channel, subfamily H, member 2
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1
KCNE2	Potassium voltage-gated channel, Isk-related family, member 2
KCNE1	Potassium voltage-gated channel, Isk-related family, member 1

5

Malignant hyperthermia

Gene	protein
RYR1	Ryanodine receptor 1 (skeletal)
CACNA1S	Calcium channel, voltage-dependent, L type, alpha 1S subunit

Metabolic myopathies

Gene	protein
GAA	Acid alpha-glucosidase preproprotein
AGL	Amylo-1,6-glucosidase, 4-alpha-glucanotransferase
GBE1	Glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)
PYGM	Glycogen phosphorylase
PFKM	Phosphofructokinase, muscle
PHKA1	Phosphorylase b kinase, alpha submit
PGM1	Phosphoglucomutase 1
GYG1	Glycogenin 1
GYS1	Glycogen synthase 3 glycogen synthase 1 (muscle) glycogen synthase 1 (muscle)
PRKAG2	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit
RBCK1	RanBP-type and C3HC4-type zinc finger containing 1 (heme-oxidized IRP2 ubiquitin ligase 1)
PGK1	Phosphoglycerate kinase 1
PGAM2	Phosphoglycerate mutase 2 (muscle)
LDHA	Lactate dehydrogenase A
ENO3	Enolase 3, beta muscle specific
CPT2	Carnitine palmitoyltransferase II
SLC22A5	Solute carrier family 22 member 5
SLC25A20	Carnitine-acylcarnitine translocase
ETFA	Electron-transfer-flavoprotein, alpha polypeptide
ETFB	Electron-transfer-flavoprotein, beta polypeptide
ETFDH	Electron-transferring-flavoprotein dehydrogenase
ACADVL	Acyl-Coenzyme A dehydrogenase, very long chain
ABHD5	Abhydrolase domain containing 5
PNPLA2	Adipose triglyceride lipase (desnutrin)
LPIN1	Lipin 1 (phosphatidic acid phosphatase 1)
PNPLA8	Patatin-like phospholipase domain containing 8

5

Hereditary Cardiomyopathies

Gene	protein
MYH6	Myosin heavy chain 6
MYH7	Myosin, heavy polypeptide 7, cardiac muscle, beta
TNNT2	Troponin T2, cardiac
TPM1	Tropomyosin 1 (alpha)
MYBPC3	Cardiac myosin binding protein-C
PRKAG2	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit
TNNI3	Troponin I, cardiac
MYL3	Myosin light chain 3
TTN	Titin
MYL2	Myosin light chain 2
ACTC1	Actin, alpha, cardiac muscle precursor
CSRP3	Cysteine and glycine-rich protein 3 (cardiac LIM protein)
TNNC1	Slow troponin C
VCL	Vinculin
MYLK2	Myosin light chain kinase 2
CAV3	Caveolin 3
MYOZ2	Myozenin 2, or calsarcin 1, a Z disk protein
JPH2	Junctophilin-2
PLN	Phospholamban
NEXN	Nexilin(F-actin binding protein)
ANKRD1	Ankyrin repeat domain 1 (cardiac muscle)
ACTN2	Actinin alpha2
NDUFAF1	NADH-ubiquinone oxidoreductase 1 alpha subcomplex
TSFM	Ts translation elongation factor, mitochondrial
AARS2	Alanyl-tRNA synthetase 2, mitochondrial
MRPL3	Mitochondrial ribosomal protein L3
COX15	COX15 homolog, cytochrome c oxidase assembly protein (yeast)
MTO1	Mitochondrial tRNA translation optimization 1
MRPL44	Mitochondrial ribosomal protein L44
LMNA	Lamin A/C
LDB3	LIM domain binding 3
SCN5A	Voltage-gated sodium channel type V alpha
DES	Desmin
EYA4	Eyes absent 4
SGCD	Delta-sarcoglycan
TCAP	Telethonin
ABCC9	ATP-binding cassette, sub-family C (member 9)
TMPO	Lamina-associated polypeptide 2
PSEN2	Presenilin 2

CRYAB	Crystallin, alpha B
FKTN	Fukutin
TAZ	Tafazzin
DMD	Dystrophin
LAMA4	Laminin alpha 4
ILK	Integrin-linked kinase
MYPN	Myopalladin
RBM20	RNA binding motif protein 20
SYNE1	Spectrin repeat containing, nuclear envelope 1 (nesprin 1)
MURC	Muscle-related coiled-coil protein
DOLK	Dolichol kinase
GATAD1	GATA zinc finger domain containing 1
SDHA	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)
GAA	Acid alpha-glucosidase preproprotein
DTNA	Dystrobrevin, alpha
FLNA	Filamin A, alpha (actin binding protein 280)
TGFB3	Transforming growth factor, beta 3
RYR2	Ryanodine receptor 2
TMEM43	Transmembrane protein 43
DSP	Desmoplakin
PKP2	Plakophilin 2
DSG2	Desmoglein 2
DSC2	Desmocollin 2
JUP	Junction plakoglobin
CASQ2	Calsequestrin 2 (cardiac muscle)
KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1
KCNH2	Voltage-gated potassium channel, subfamily H, member 2
ANK2	Ankyrin 2
KCNE1	Potassium voltage-gated channel, Isk-related family, member 1
KCNE2	Potassium voltage-gated channel, Isk-related family, member 2
KCNJ2	Potassium inwardly-rectifying channel J2
CACNA1C	Calcium channel, voltage-dependent, L type, alpha 1C subunit
SCN4B	Sodium channel, voltage-gated, type IV, beta subunit
AKAP9	A kinase (PRKA) anchor protein (yotiao) 9
SNTA1	Syntrophin, alpha 1
KCNJ5	Potassium inwardly-rectifying channel, subfamily J, member 5
NPPA	Natriuretic peptide precursor A
KCNA5	Potassium voltage-gated channel, shaker-related subfamily, member 5
GJA5	Connexin 40
SCN1B	Sodium channel, voltage-gated, type I, beta subunit

SCN2B	Sodium channel, voltage-gated, type II, beta subunit
NUP155	Nucleoporin 155 kDa
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like
CACNB2	Calcium channel, voltage-dependent, beta 2 subunit
KCNE3	Potassium voltage-gated channel, Isk-related family, member 3
SCN3B	Sodium channel, voltage-gated, type III, beta subunit
HCN4	Hyperpolarization activated cyclic nucleotide-gated potassium channel 4

Congenital myasthenic syndromes

Gene	**protein**
CHRNA1	Cholinergic receptor, nicotinic, alpha polypeptide 1
CHRNB1	Cholinergic receptor, nicotinic, beta 1 muscle
CHRND	Cholinergic receptor, nicotinic, delta
CHRNE	Cholinergic receptor, nicotinic, epsilon
RAPSN	Rapsyn
CHAT	Choline acetyltransferase isoform
COLQ	Acetylcholinesterase collagen-like tail subunit
MUSK	muscle, skeletal, receptor tyrosine kinase
DOK7	Docking protein 7
AGRN	Agrin
GFPT1	Glutamine-fructose-6-phosphate transaminase 1
DPAGT1	Dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)
LAMB2	Laminin, beta 2 (laminin S)
SCN4A	Sodium channel, voltage-gated, type IV, alpha
CHRNG	Cholinergic receptor, nicotinic, gamma polypeptide
PLEC	plectin
ALG2	Alpha-1,3/1,6-mannosyltransferase
ALG14	UDP-N-acetylglucosaminyltransferase
SYT2	Synaptotagmin II
PREPL	Prolyl endopeptidase-like

Motor Neuron diseases

Gene	**protein**
SMN1	Survival of motor neuron 1, telomeric
IGHMBP2	Immunoglobulin mu binding protein 2
PLEKHG5	Pleckstrin homology domain containing, family G (with RhoGef domain) member 5
HSPB8	Heat shock 27kDa protein 8
HSPB1	Heat shock 27kDa protein 1

HSPB3	Heat shock 27kDa protein 3
AARS	Alanyl-tRNA synthetase
GARS	Glycyl-tRNA synthetase
BSCL2	Seipin
REEP1	Receptor accessory protein 1
SLC5A7	Solute carrier family 5 (sodium/choline cotransporter), member 7
DCTN1	Dynactin 1
UBA1	Ubiquitin-activating enzyme 1
ATP7A	ATPase, Cu++ transporting, alpha polypeptide
DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2
TRPV4	Transient receptor potential cation channel, subfamily V, member 4
DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1
BICD2	Bicaudal D homolog 2 (Drosophila)
FBXO38	F-box protein 38
ASAH1	N-acylsphingosine amidohydrolase (acid ceramidase) 1
VAPB	Vesicle-associated membrane protein-associated protein B and C
EXOSC8	Exosome component 8
SOD1	Superoxide dismutase 1, soluble
ALS2	Alsin
SETX	Senataxin
FUS	Fusion (involved in t(12;16) in malignant liposarcoma)
ANG	Angiogenin
TARDBP	TAR DNA binding protein
FIG4	Sac domain-containing inositol phosphatase 3
OPTN	Optineurin
ATXN2	Ataxin 2
VCP	Valosin-containing protein
UBQLN2	Ubiquilin 2
SIGMAR1	Sigma non-opioid intracellular receptor 1
CHMP2B	Charged multivesicular body protein 2B
PFN1	Profilin 1
MATR3	Matrin 3
NEFH	Neurofilament, heavy polypeptide
PRPH	Peripherin
C9orf72	Chromosome 9 open reading frame 72
CHCHD10	Coiled-coil-helix-coiled-coil-helix domain containing 10
SQSTM1	Sequestosome 1
AR	Androgen receptor
GLE1	GLE1 RNA export mediator homolog (yeast)
ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
PIP5K1C	Phosphatidylinositol-4-phosphate 5-kinase, type I, gamma

EXOSC3	Exosome component 3
VRK1	Vaccinia related kinase 1
SLC52A3	Solute carrier family 52, riboflavin transporter, member 3
SLC52A2	Solute carrier family 52, riboflavin transporter, member 2
HEXB	Hexosaminidase B

Hereditary motor and sensory neuropathies

Gene	**Protein**
PMP22	Peripheral myelin protein 22
MPZ	Myelin protein zero
LITAF	Lipopolysaccharide-induced TNF factor
EGR2	Early growth response 2 protein
NEFL	Neurofilament, light polypeptide 68kDa
HOXD10	Homeobox D10
ARHGEF10	Rho guanine nucleotide exchange factor 10
FBLN5	Fibulin 5 (extra-cellular matrix)
DNM2	Dynamin 2
YARS	Tyrosyl-tRNA synthetase
INF2	Inverted formin 2
GNB4	Guanine nucleotidebinding protein, beta-4
GDAP1	Ganglioside-induced differentiation-associated protein 1
MTMR2	Myotubularin-related protein 2
SBF2	SET binding factor 2
SBF1	SET binding factor 1
SH3TC2	KIAA1985 protein
NDRG1	N-myc downstream regulated gene 1
PRX	Periaxin
HK1	Hexokinase 1
FGD4	Actin-filament binding protein Frabin
FIG4	Sac domain-containing inositol phosphatase 3
SURF1	surfeit 1
GJB1	Gap junction protein, beta 1, 32kDa (connexin 32)
AIFM1	Apoptosis-inducing factor, mitochondrionassociated 1
PRPS1	Phosphoribosyl pyrophosphate synthetase 1
PDK3	Pyruvate dehydrogenase kinase, isoenzyme 3
KIF1B	Kinesin family member 1B
MFN2	Mitofusin 2
RAB7A	RAB7, member RAS oncogene family
TRPV4	Transient receptor potential cation channel, subfamily V, member 4
GARS	Glycyl-tRNA synthetase

HSPB1	Heat shock 27kDa protein 1
HSPB8	Heat shock 27kDa protein 8
AARS	Alanyl-tRNA synthetase
DYNC1H1	Dynein, cytoplasmic 1, heavy chain 1
LRSAM1	leucine rich repeat and sterile alpha motif containing 1
DHTKD1	dehydrogenase E1 and transketolase domain containing 1
TRIM2	Tripartite motif containing 2
TFG	TRK-fused gene
MARS	methionyl-tRNA synthetase
KIF5A	Kinesin family member 5A
LMNA	Lamin A/C
MED25	Mediator complex subunit 25
DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2
HINT1	Histidine triad nucleotide binding protein 1
KARS	Lysyl-tRNA synthetase
PLEKHG5	Pleckstrin homology domain containing, family G (with RhoGef domain) member 5
COX6A1	Cytochrome c oxidase subunit VIa polypeptide 1
IGHMBP2	Immunoglobulin mu binding protein 2
SPTLC1	Serine palmitoyltransferase subunit 1
SPTLC2	Serine palmitoyltransferase long chain base subunit 2
ATL1	Atlastin GTPase 1
KIF1A	Kinesin family member 1A
WNK1	WNK lysine deficient protein kinase 1
IKBKAP	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
NGF	Nerve growth factor (beta polypeptide)
DNMT1	DNA (cytosine-5)-methyltransferase 1
SLC12A6	Potassium chloride cotransporter KCC3
GJB3	Gap junction protein, beta 3, 31kDa (=connexin 31)
sept-09	Septin 9
GAN	Gigaxonin
CTDP1	CTD phosphatase subunit 1
VRK1	Vaccinia related kinase 1

Hereditary paraplegia

Gene symbol	protein
ATL1	Atlastin
SPAST	Spastin
NIPA1	Non-imprinted in Prader-Willi/Angelman syndrome 1
KIAA0196	Strumpellin
KIF5A	Kinesin family member 5A
RTN2	Reticulon 2
HSPD1	Heat shock 60kDa protein 1 (chaperonin)
BSCL2	Seipin
REEP1	Receptor accessory protein 1
ZFYVE27	Protrudin
SLC33A1	Solute carrier family 33 (acetyl- CoA transporter)
CYP7B1	Cytochrome P450, family 7, subfamily B, polypeptide 1
SPG7	Paraplegin
SPG11	Spatacsin
ZFYVE26	Spastizin
ERLIN2	ER lipid raft associated 2
SPG20	Spartin
SPG21	Maspardin
B4GALNT1	beta-1,4-N-acetyl-galactosaminyl transferase 1
DDHD1	DDHD domain containing 1
KIF1A	Kinesin family member 1A
FA2H	Fatty acid 2-hydroxylase
PNPLA6	Patatin-like phospholipase domain containing 6
C19orf12	chromosome 19 open reading frame 12
GJC2	gap junction protein, gamma 2, 47kDa
NT5C2	5'-nucleotidase, cytosolic II
GBA2	glucosidase, beta (bile acid) 2
AP4B1	adaptor-related protein complex 4, beta 1 subunit
AP5Z1	Hypothetical protein LOC9907
TECPR2	tectonin beta-propeller repeat containing 2
AP4M1	Adaptor-related protein complex 4, mu 1 subunit
AP4E1	Adaptor-related protein complex 5, zeta 1 subunit
AP4S1	adaptor-related protein complex 4, sigma 1 subunit
DDHD2	DDHD domain containing 2
C12orf65	adaptor-related protein complex 4, sigma 1 subunit
CYP2U1	cytochrome P450, family 2, subfamily U, polypeptide 1
ARL6IP1	ADP-ribosylation factor-like 6 interacting protein 1
AMPD2	adenosine monophosphate deaminase 2
ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1

ALDH3A2	Aldehyde dehydrogenase 3A2
ALS2	Alsin
L1CAM	L1 cell adhesion molecule
PLP1	Proteolipid protein 1
MTPAP	mitochondrial poly(A) polymerase
AFG3L2	AFG3 ATPase family gene 3-like 2 (S. cerevisiae) 1
SACS	Sacsin

Other neuromuscular disorders

Gene	**protein**
TOR1A	Torsin A
SGCE	Sarcoglycan, epsilon
IKBKAP	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein
TTR	Transthyretin (prealbumin, amyloidosis type I)
KIF21A	Kinesin family member 21A
PHOX2A	Paired-like aristaless homeobox protein 2A
TUBB3	Tubulin, beta 3
TPM2	Tropomyosin 2 (beta)
MYH3	Myosine, heavy chain 3, skeletal muscle, embryonic
TNNI2	Troponin I, type 2
TNNT3	Troponin T3, skeletal
SYNE1	Spectrin repeat containing, nuclear envelope 1 (nesprin 1)
MYH8	Myosin heavy chain, 8, skeletal muscle, perinatal
POLG	Polymerase (DNA directed), gamma
SLC25A4	Mitochondrial carrier; adenine nucleotide translocator
C10orf2	chromosome 10 open reading frame 2
POLG2	Mitochondrial DNA polymerase, accessory subunit
RRM2B	Ribonucleotide reductase M2 B (TP53 inducible)
TK2	Thymidine kinase 2, mitochondrial
SUCLA2	Succinate-CoA ligase, ADP-forming, beta subunit
OPA1	optic atrophy 1
STIM1	Stromal interaction molecule 1
ORAI1	ORAI calcium release-activated calcium modulator 1
PUS1	Pseudouridylate synthase 1
CHCHD10	Coiled-coil-helix-coiled-coil-helix domain containing 10
CASQ1	Calsequestrin 1 (fast-twitch, skeletal muscle)
YARS2	tyrosyl-tRNA synthetase 2, mitochondrial

Any one of the above listed genes may be targeted in replacement gene therapy, wherein the gene of interest is a functional version of the deficient or mutated gene.

Alternatively, the above listed genes may be used as target for gene editing. Gene editing is used to correct the sequence of a mutated gene or modify the expression or regulation of a deficient/abnormal gene so that a functional gene is expressed in muscle cells. In such cases, the gene of interest is chosen from those encoding therapeutic RNAs such as interfering RNAs, guide RNAs for genome editing and antisense RNAs capable of exon skipping, wherein the therapeutic RNAs target the preceding list of genes. Tools such as CRISPR/Cas9 may be used for that purpose.

In some embodiments, the target gene for gene therapy (additive gene therapy or gene editing) is a gene responsible for one of the muscular dystrophies listed above, in particular DMD (*DMD*, *BMD* genes); LGMDs (*CAPN3* gene and others); Facio-scapulo-humeral dystrophies, type 1 (FSHD1A; *DUX4* or *FRG1* gene) and type 2 (FSHD1B; *SMCHD1* gene) and titinopathies (*TTN* gene).

In some embodiments, the pharmaceutical composition of the invention is for use for treating muscular diseases (i.e., myopathies) or muscular injuries, in particular neuromuscular genetic disorders, with no liver damage, such as for example : Muscular dystrophies, Congenital muscular dystrophies, Congenital myopathies, Distal myopathies, Other myopathies, Myotonic syndromes, Ion Channel muscle diseases, Malignant hyperthermia, Metabolic myopathies, Hereditary Cardiomyopathies, Congenital myasthenic syndromes, Motor Neuron diseases, Hereditary paraplegia, Hereditary motor and sensory neuropathies and other neuromuscular disorders.

Muscular dystrophies include in particular:

- Dystrophinopathies, a spectrum of X-linked muscle diseases caused by pathogenic variants in *DMD* gene, which encodes the protein dystrophin. Dystrophinopathies comprises Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD) and DMD-associated dilated cardiomyopathy;

- The Limb-girdle muscular dystrophies (LGMDs) which are a group of disorders that are clinically similar to DMD but occur in both sexes as a result of autosomal

recessive and autosomal dominant inheritance. Limb-girdle dystrophies are caused by mutation of genes that encode sarcoglycans and other proteins associated with the muscle cell membrane, which interact with dystrophin. The term LGMD1 refers to genetic types showing dominant inheritance (autosomal dominant), whereas LGMD2 refers to types with autosomal recessive inheritance. Pathogenic variants at more than 50 loci have been reported (LGMD1A to LGMD1H; LGMD2A to LGMD2Y). Calpainopathy (LGMD2A) is caused by mutation of the gene *CAPN3* with more than 450 pathogenic variants described;

- The Emery-Dreifuss Muscular Dystrophy (EDMD) caused by defects in one of the gene including the *EMD* gene (coding for emerin), the *FHL1* gene and the *LMNA* gene (encoding lamin A and C);

- Nesprin-1 and Nesprin-2 related muscular dystrophy caused by defects in the *SYNE1* and *SYNE2* gene, respectively; LUMA related muscular dystrophy caused by defects in the *TMEM43* gene; LAP1B related muscular dystrophy caused by defects in the *TOR1AIP1* gene; and

- Facio-scapulo-humeral muscular dystrophy, type 1 (FSHD1A), such as associated with defect in the *DUX4* gene (contraction of the D4Z4 macrosatellite repeat in the subtelomeric region of chromosome 4q35) or the *FRG1* gene; Facio-scapulo-humeral muscular dystrophy, type 2 (FSHD1B) caused by defects in the *SMCHD1* gene.

A specific example of gene editing would be the treatment of Limb-girdle muscular dystrophy 2A (LGMD2A) which is caused by mutations in the calpain-3 gene (*CAPN3*). Other examples would be the treatment of mutations in the DMD or TNT genes.

Thus, by gene editing or gene replacement a correct version of this gene is provided in muscle cells of affected patients, this may contribute to effective therapies against this disease. Other genetic diseases of the muscle as listed above could be treated by gene replacement or gene editing using the same principle.

Replacement or additive gene therapy may be used to treat cancer, in particular rhabdomyosarcomas. Genes of interest in cancer could regulate the cell cycle or the

metabolism and migration of the tumor cells, or induce tumor cell death. For instance, inducible caspase-9 could be expressed in muscle cells to trigger cell death, preferably in combination therapy to elicit durable anti-tumor immune responses.

Gene editing may be used to modify gene expression in muscle cells, in the case of auto-immunity or cancer, or to perturb the cycle of viruses in such cells. In such cases, preferably, the gene of interest is chosen from those encoding guide RNA (gRNA), site-specific endonucleases (TALEN, meganucleases, zinc finger nucleases, Cas nuclease), DNA templates and RNAi components, such as shRNA and microRNA. Tools such as CRISPR/Cas9 may be used for this purpose.

In some embodiments, gene therapy is used for treating diseases affecting other tissues, by expression of a therapeutic gene in muscle tissue. This is useful to avoid expression of the therapeutic gene in the liver, in particular in patients having a concurrent hepatic disorder such as hepatitis. The therapeutic gene encodes preferably a therapeutic protein, peptide or antibody which is secreted from the muscle cells into the blood stream where it can be delivered to other target tissues such as for example the liver. Examples of therapeutic genes include with no limitation: Factor VIII, Factor IX and GAA genes.

The pharmaceutical composition of the invention which comprises AAV vector particles with reduced liver tropism may be administered to patients having concurrent liver disease such as for example hepatitis including viral or toxic hepatitis.

In the context of the invention, a therapeutically effective amount refers to a dose sufficient for reversing, alleviating or inhibiting the progress of the disorder or condition to which such term applies, or reversing, alleviating or inhibiting the progress of one or more symptoms of the disorder or condition to which such term applies.

The effective dose is determined and adjusted depending on factors such as the composition used, the route of administration, the physical characteristics of the individual under consideration such as sex, age and weight, concurrent medication, and other factors, that those skilled in the medical arts will recognize.

In the various embodiments of the present invention, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and/or vehicle.

A "pharmaceutically acceptable carrier" refers to a vehicle that does not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

Preferably, the pharmaceutical composition contains vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or suspensions. The solution or suspension may comprise additives which are compatible with viral vectors and do not prevent viral vector particle entry into target cells. In all cases, the form must be sterile and must be fluid to the extent that easy syringe ability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. An example of an appropriate solution is a buffer, such as phosphate buffered saline (PBS) or Ringer lactate.

The invention provides also a method for treating a disease affecting muscle tissue in particular skeletal muscle tissue and/or cardiac tissue, comprising: administering to a patient a therapeutically effective amount of the pharmaceutical composition as described above.

The invention provides also a method for treating a disease by expression of a therapeutic gene in muscle tissue, comprising: administering to a patient a therapeutically effective amount of the pharmaceutical composition as described above.

As used herein, the term “patient” or “individual” denotes a mammal. Preferably, a patient or individual according to the invention is a human.

In the context of the invention, the term "treating" or "treatment", as used herein, means reversing, alleviating or inhibiting the progress of the disorder or condition to which such

term applies, or reversing, alleviating or inhibiting the progress of one or more symptoms of the disorder or condition to which such term applies.

The pharmaceutical composition of the present invention, is generally administered according to known procedures, at dosages and for periods of time effective to induce a therapeutic effect in the patient.

The administration may be parenteral, oral, local, or loco-regional. The parenteral administration is advantageously by injection or perfusion, such as e subcutaneous (SC), intramuscular (IM), intravascular such as intravenous (IV), intraperitoneal (IP), intradermal (ID) or else. Preferably, the administration produces a systemic effect in the whole body, *i.e.*, all the muscles of the patient, including the diaphragm and the heart. Preferably, the administration is systemic, more preferably parenteral.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques, which are within the skill of the art. Such techniques are explained fully in the literature.

The invention will now be exemplified with the following examples, which are not limitative, with reference to the attached drawings in which:

FIGURE LEGENDS

- <u>Figure 1:</u> Design of new hybrid AAV serotypes between AAV9 and AAVrh74. .

A. Cap genes (VP1) of AAV9 and AAVrh74 highlighting the sequence of the variable region. The variable region N-term and C-term sequences are SEQ ID NO: 9 and SEQ ID NO: 10 for AAV9 and SEQ ID NO: 11 and SEQ ID NO: 12 for AAVrh74. **B**. Hybrid AAV9-rh74 and hybrid AAVrh74-9 Cap genes (VP1) .

- <u>Figure 2:</u> Productions of new hybrid AAV serotypes between AAV9 and AAVrh74

AAV9-rh74 and AAVrh74-9 hybrid serotypes and controls (AAV9, AAVrh74) were produced in HEK293T cells. Viral genomes were quantified by Taqman real-time PCR. Error bars represent SEM.

- Figure 3: Design of biodistribution study.

Vg: viral genome.

- Figure 4: Quantification of transgene expression in muscles and organs following systemic administration of AAV hybrid serotypes.

Luciferase expression was quantified in skeletal muscles (**A** and **B**) and organs (**C** and **D**) of mice injected (with low dose = 2 E10 vg/mouse (**A** and **C**) or high dose = 1 E11 vg/mouse (**B** and **D**) of AAV9-rh74 and AAVrh74-9 hybrid serotypes and controls (AAV9, AAVrh74). Error bars represent SEM. TA: Tibialis anterior. Pso: Psoas. Qua: Quadriceps. Dia: Diaphragm. RLU: relative light units.

EXAMPLE 1: Design and production of hybrid rAAV serotype vectors with AAV9-rh74 and rh74-AAV9 capsids

1. Material and Methods

Plasmid construction for new serotypes

To construct a plasmid containing AAV2 Rep sequence and Hybrid Cap 9-rh74, a fragment of 1029 nt, containing the highly variable part of AAV-rh74 Cap flanked with AAV9 Cap sequence fragments and restriction sites BsiWI in 5' and Eco47III in 3', was synthesized (GENEWIZ). This fragment was then inserted using the mentioned restriction sites in the plasmid pAAV2-9, which contains AAV2 Rep and AAV9 Cap, to replace the AAV9 Cap corresponding sequence.

To construct a plasmid containing AAV2 Rep sequence and Hybrid Cap rh74-9, a fragment of 2611 nt, containing the highly variable part of AAV-9 Cap flanked with the rest of AAV_rh74 Cap sequence, a part of AAV2 Rep sequence and restriction sites, HindIII in 5' and PmeI in 3', was synthesized (GENEWIZ). This fragment was then inserted using the mentioned restriction sites in the plasmid pAAV2-9, which contains AAV2 Rep and AAV9 Cap, to replace the full AAV9 Cap sequence.

AAV production

Two protocols, corresponding to two scales of production, were used in this study. In the miniscale condition, adherent HEK293 are grown in DMEM added with 10% fetal bovine serum (FBS), in multiwell-6 plates. In the upper scale condition, HEK293T are grown in suspension in 250 mL of serum-free medium. The cells are transfected with 3 plasmids: i) a

transgene plasmid, containing AAV2 ITRs flanking an expression cassette coding for the firefly luciferase, ii) the helper plasmid pXX6, containing adenoviral sequences necessary for AAV production, and iii) a plasmid containing AAV Rep and Cap genes, defining the serotype of AAV. Two days after transfection, the cells are lysed to liberate the AAV particles.

The viral lysate is purified through two rounds of Cesium Chloride density gradient ultracentrifugation followed by dialysis or by affinity chromatography. Viral genomes are quantified by a TaqMan real-time PCR assay using primers and probes corresponding to the ITRs of the AAV vector genome (Rohr et al., J. Virol. Methods, 2002, 106, 81–88).

2. Results

Design of new serotypes

The amino acid sequences of AAV9 (SEQ ID NO: 1) and AAV-rh74 (SEQ ID NO: 2) VP1 protein (encoded by the Cap genes) were aligned using Blastp, and a highly variable region was detected, ranging from amino acid position 449 to position 609 in AAV9 Cap, and from position 450 to position 611 in AAV-rh74 Cap (**- Figure *1*A**). Then two new Cap genes (SEQ ID NO: 5 and SEQ ID NO: 7) were constructed by replacement of the highly variable region of each serotype by the other (**- Figure *1*B**). These two hybrid Cap genes were inserted into a plasmid containing the AAV2 Rep sequence, allowing production of recombinant AAV particles. The new serotypes were named "Hybrid AAV 9-rh74" (SEQ ID NO: 3) for the one containing AAV9 cap sequence for its major part, and the AAV-rh74 highly variable part, and "Hybrid AAV rh74-9" (SEQ ID NO: 4) for the one containing AAVrh74 Cap sequence for its major part, and the AAV9 highly variable part.

Production of the new hybrid AAV serotypes

AAV production was performed at two different scales with the new hybrid serotypes and controls (2mL in 6-well plate or a 250 mL culture in suspension). As shown in **- Figure *2***, the new hybrid serotypes can be produced with a yield suitable for gene transfer applications.

EXAMPLE 2: Biodistribution study of hybrid AAV serotype vectors with AAV9-rh74 and AAVrh74-9 capsids

1. Material and Methods

In vivo experiments

The AAV vectors were administered to one month-old B6Albino male mice, by intravenous injection in the tail vein. Two doses were assessed, a low dose of 2 E10 (2.10^{10}) viral genomes (vg) /mouse, and a high dose of 1 E11 (10^{11}) vg /mouse. Fifteen days after injection, luciferase imaging was performed using IVIS Lumina device (PERKIN ELMER) on mice previously anesthetized (ketamine + xylazine) and injected intraperitonally by luciferin. Thirty days after injection, mice were sacrificed and skeletal muscles and organs were sampled and frozen in liquid nitrogen.

Molecular analysis

Samples were homogenized in Lysis buffer [Tris-base 25mM, $MgCl_2$ 8mM, DTT 1mM, EDTA 1mM, glycerol 15%, Triton X-100 0.2%] supplemented with Protease Inhibitor Cocktail (Roche). Luciferase expression quantification was performed on sample lysates using Enspire multimode plate reader (Roche), in Assay Buffer [Tris-base 25mM, $MgCl_2$ 8mM, DTT 1mM, EDTA 1mM, glycerol 15%, ATP 2mM] extemporaneously supplemented with luciferin at 83µM. The total amount of protein in samples was measured using Pierce BCA protein assay kit (Thermo Fisher). The result of luciferase luminescence was normalized by the total protein amount.

For quantification of viral genomes in samples (VCN for Vector Copy Number), DNA was extracted from samples using NucleoSpin Tissue (Macherey-Nagel). Real-time PCR was performed on 100 ng of DNA, using the same protocol as described above for AAV vectors titration. Exon Mex5 of titin gene was amplified in the same experiment to be used as genomic control.

2. Results

To assess the biodistribution of the new serotypes, Hybrid AAV and control vectors containing an expression cassette encoding the luciferase reporter gene under the control of the ubiquitous CMV promoter were produced. The vectors were administered to mice at two doses (low dose = 2 E10 vg/mouse; high dose = 1 E11 vg/mouse), by systemic injection.

Whole body imaging was performed 15 days after administration, and different skeletal muscles and organs were sampled after one month of expression (**Figure 3**).

After sampling, luciferase expression in muscles and other organs was quantified then normalized by total protein amount in sample, in different skeletal muscles and organs.

In muscle, hybrid AAV 9-74 allowed a good level of transgene expression in all tested muscles including skeletal and cardiac muscles, similarly to AAVrh74 (**Figure 4A to 4D**). Surprisingly, both hybrids have a drastically reduced transgene expression in liver, compared to the high level of transgene expression of the AAV9 or AAV-rh74 controls (**Figure 4C and 4D**).

Two new serotypes were generated using a combination of AAV9 and AAV-rh74, two serotypes that efficiently infect the muscle tissue but also the liver. The resulting hybrids show gene transfer in skeletal muscle, without efficient transduction of the liver. These hybrid serotypes are therefore of interest when transduction of skeletal muscle but not liver is needed.

CLAIMS:

1. A recombinant adeno-associated virus (AAV) capsid protein, which is a hybrid between AAV serotype 9 (AAV9) and AAV serotype 74 (AAVrh74) capsid proteins, wherein said recombinant hybrid AAV capsid protein has a reduced liver tropism compared to the parent AAV9 and AAVrh74 capsid proteins, and wherein said recombinant hybrid AAV capsid protein comprises a sequence selected from the group consisting of the sequences SEQ ID NO: 3 and SEQ ID NO: 4 and the sequences having at least 85% identity with said sequences, preferably SEQ ID NO:3.

2. The recombinant hybrid AAV capsid protein according to claim 1, which has a muscle tropism similar to that of the parent AAV9 and/or AAVrh74 capsid proteins.

3. The recombinant hybrid AAV capsid protein according to claim 1 or claim 2, which results from the replacement of a variable region in the AAV9 or AAVrh74 capsid sequence with the corresponding variable region of the other AAV serotype capsid sequence,

wherein the variable region of AAV9 capsid corresponds to the sequence situated from any one of positions 331 to 493 to any one of positions 556 to 736 in AAV9 capsid of SEQ ID NO: 1 or a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 consecutive amino acids of the sequence situated from positions 493 to 556 in AAV9 capsid of SEQ ID NO: 1, and

the variable region of AAVrh74 capsid corresponds to the sequence situated from any one of positions 332 to 495 to any one of positions 558 to 738 in AAVrh74 capsid of SEQ ID NO: 2 or a fragment of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 consecutive amino acids of the sequence situated from positions 495 to 558 in AAVrh74 capsid of SEQ ID NO: 2.

4. The recombinant hybrid protein according to claim 3, wherein the recombinant hybrid AAV capsid protein results from the replacement of the variable region corresponding to the sequence situated from positions 449 to 609 in AAV9 capsid of SEQ ID NO: 1 or from positions 450 to 611 in AAVrh74 capsid of SEQ ID NO: 2 with the corresponding variable region of the other AAV serotype capsid sequence.

2019249890 26 Sep 2024

5. The recombinant hybrid AAV capsid protein according to any one of claims 1 to 4, which comprises a sequence selected from the group consisting of the sequences having at least 90%, 95%, 97%, 98% or 99% identity with said sequences SEQ ID NO: 3 and SEQ ID NO: 4, preferably which comprises a sequence selected from the group consisting of the sequences of SEQ ID NO: 3 and the sequences having at least 90%, 95%, 97%, 98% or 99% identity with said sequence.

6. The recombinant hybrid AAV capsid protein according to any one of claims 1 to 5, which comprises the sequence of SEQ ID NO: 3

7. The recombinant hybrid AAV capsid protein according to any one of claims 1 to 6, which is a hybrid VP1, VP2 or VP3 protein.

8. A recombinant chimeric AAV capsid protein, which is selected from the group consisting of:

- a chimeric VP1 protein comprising: (i) a VP1-specific N-terminal region having a sequence from natural or artificial AAV serotype other than AAV9 and AAVrh74, (ii) a VP2-specific N-terminal region having a sequence from AAV9, AAVrh74 or natural or artificial AAV serotype other than AAV9 and AAVrh74, and (iii) a VP3 C-terminal region having the sequence of a hybrid VP3 protein according to claim 7, and

- a chimeric VP2 protein comprising: (i) a VP2-specific N-terminal region having a sequence from natural or artificial AAV serotype other than AAV9 and AAVrh74, and (ii) a VP3 C-terminal region having the sequence of a hybrid VP3 protein according to claim 7.

9. A polynucleotide encoding the recombinant hybrid AAV capsid protein according to any one of claims 1 to 7 or the recombinant chimeric AAV capsid protein according to claim 8, in expressible form, and eventually further encoding AAV Replicase protein in expressible form.

10. A recombinant plasmid comprising the polynucleotide of claim 9.

11. An AAV vector particle packaging a gene of interest, which comprises the hybrid recombinant AAV capsid protein according to any one of claims 1 to 7, and/or the recombinant

chimeric AAV capsid protein according to claim 8, and eventually also at least one AAV capsid protein from natural or artificial AAV serotype other than AAV9 and AAVrh74.

12. The AAV vector particle according to claim 11, wherein the gene of interest is selected from the group consisting of:

(i) therapeutic genes;

(ii) genes encoding therapeutic proteins or peptides such as therapeutic antibodies or antibody fragments and genome editing enzymes; and

(iii) genes encoding therapeutic RNAs such as interfering RNAs, guide RNAs for genome editing and antisense RNAs capable of exon skipping.

13. A pharmaceutical composition comprising a therapeutically effective amount of AAV vector particles according to claim 11 or claim 12.

14. The pharmaceutical composition according to claim 13, when used as a medicament in gene therapy.

15. A method of gene therapy comprising administering to a patient a therapeutically effective amount of the pharmaceutical composition according to claim 13.

16. Use of AAV vector particles according to claim 11 or claim 12, or the pharmaceutical composition according to claim 13, in the manufacture of a medicament for providing gene therapy to a patient.

17. The pharmaceutical composition according to claim 13 or 14, the method according to claim 15, or the use according to claim 16, wherein the gene therapy is for treating genetic diseases, cancer or auto-immune diseases affecting muscle tissues.

18. The pharmaceutical composition according to claim 13 or 14, the method according to claim 15, or the use according to claim 16, wherein the gene therapy targets a gene responsible for a neuromuscular genetic disorders selected from the group comprising: Dystrophinopathies, Limb-girdle muscular dystrophies, Facio-scapulo-humeral dystrophies and titinopathies.

19. The pharmaceutical composition, method or use according to claim 18, wherein the target gene is selected from the group comprising: *DMD*, *BMD*, *CAPN3*, *DUX4*, *FRG1*, *SMCHD1* and TTN genes.

Genethon
INSERM (Institut National de la Santé et de la Recherche Médicale)
Universite d'Evry Val d'Essonne
Sorbonne Universite
Association Institut de Myologie

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

FIGURE 1

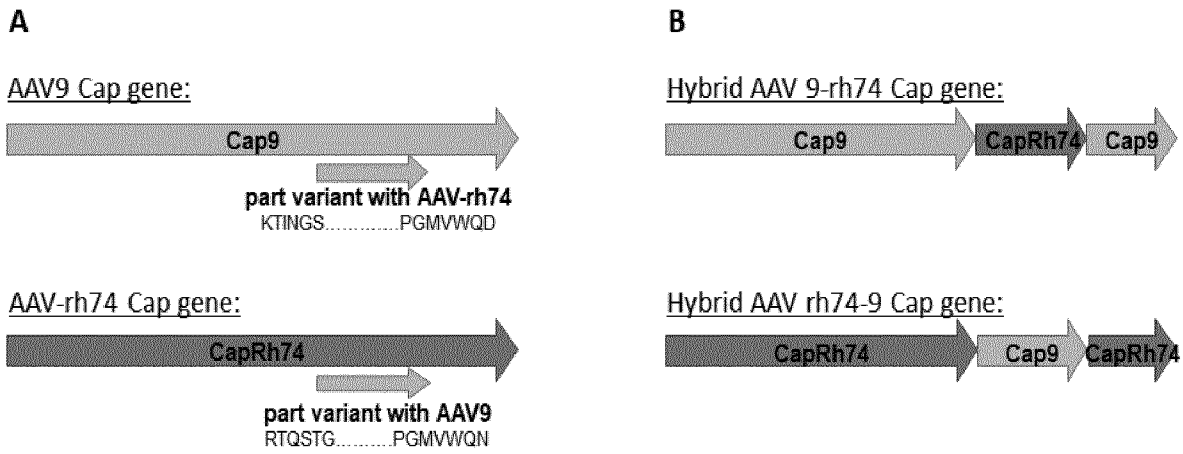
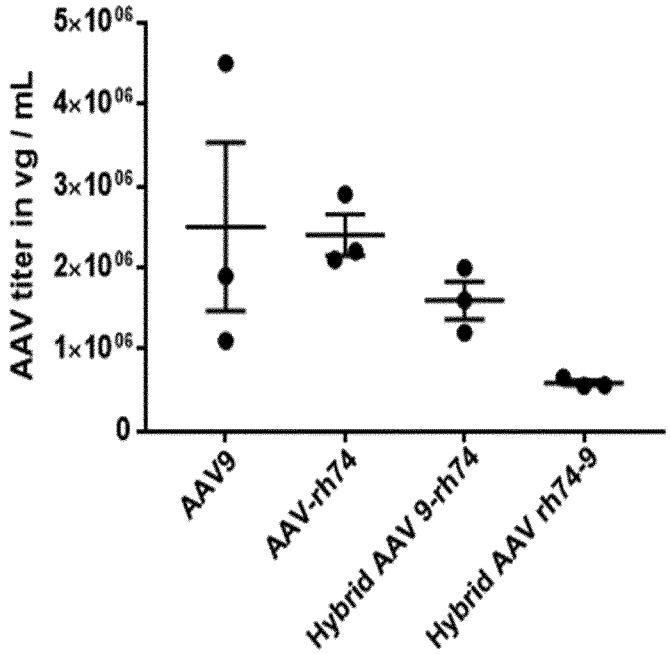


FIGURE 2



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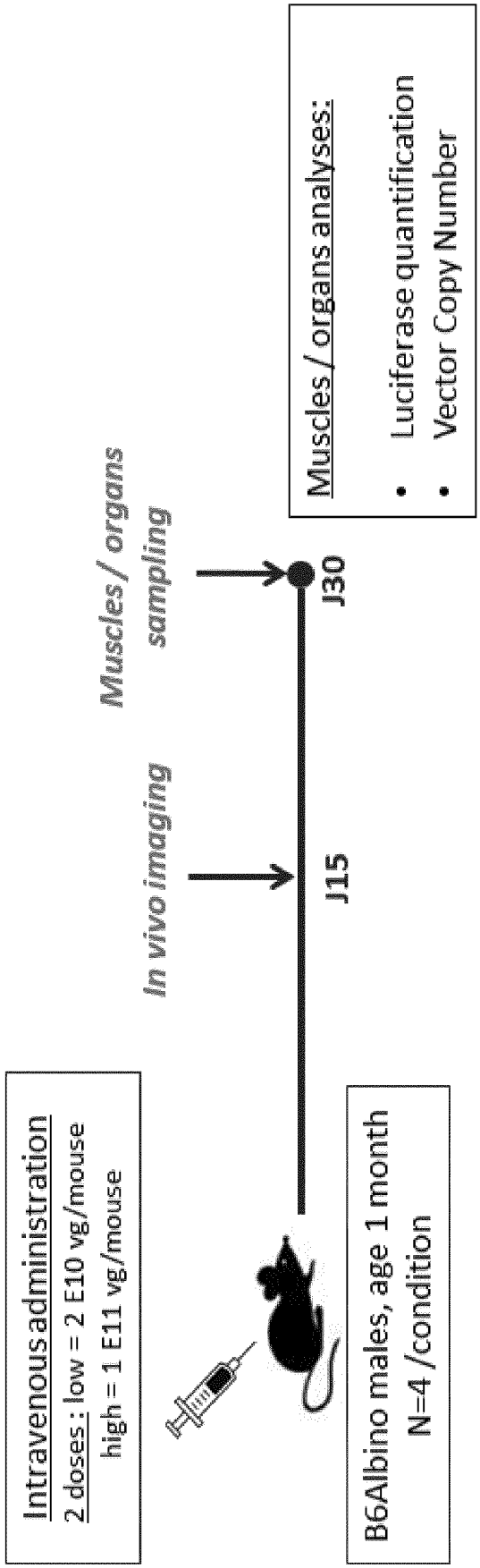
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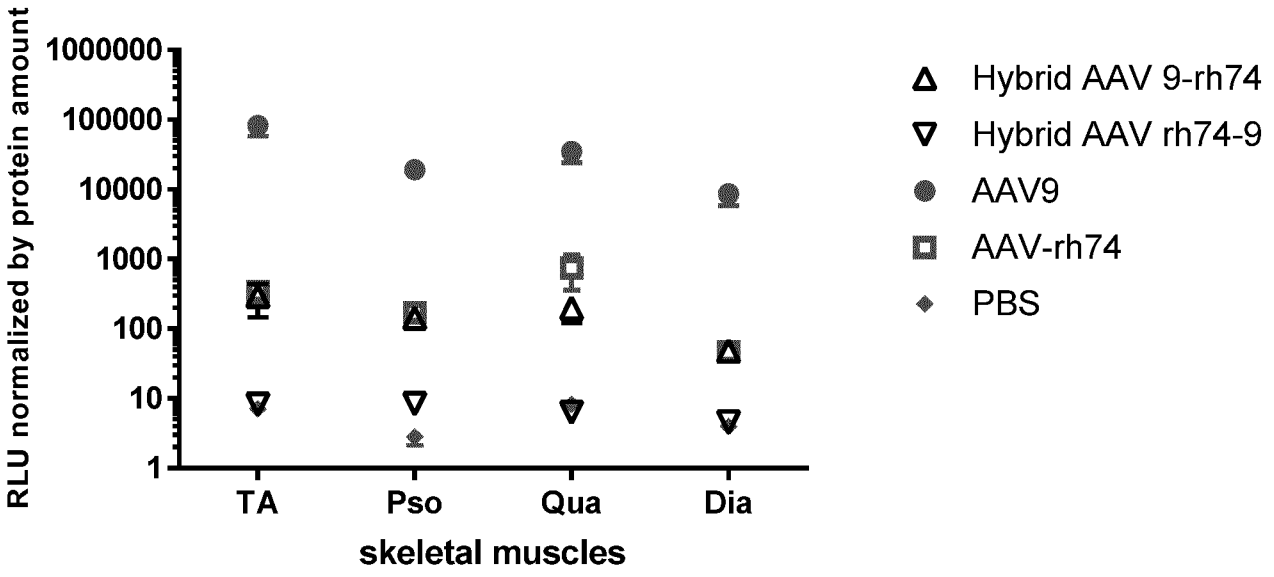
Luciferase quantification
Vector Copy Number

FIGURE 4

A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10000
1000
100
10
1
TA
Pso
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Dia
skeletal muscles
Hybrid AAV 9-rh74
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AAV9
AAV-rh74
PBS

B

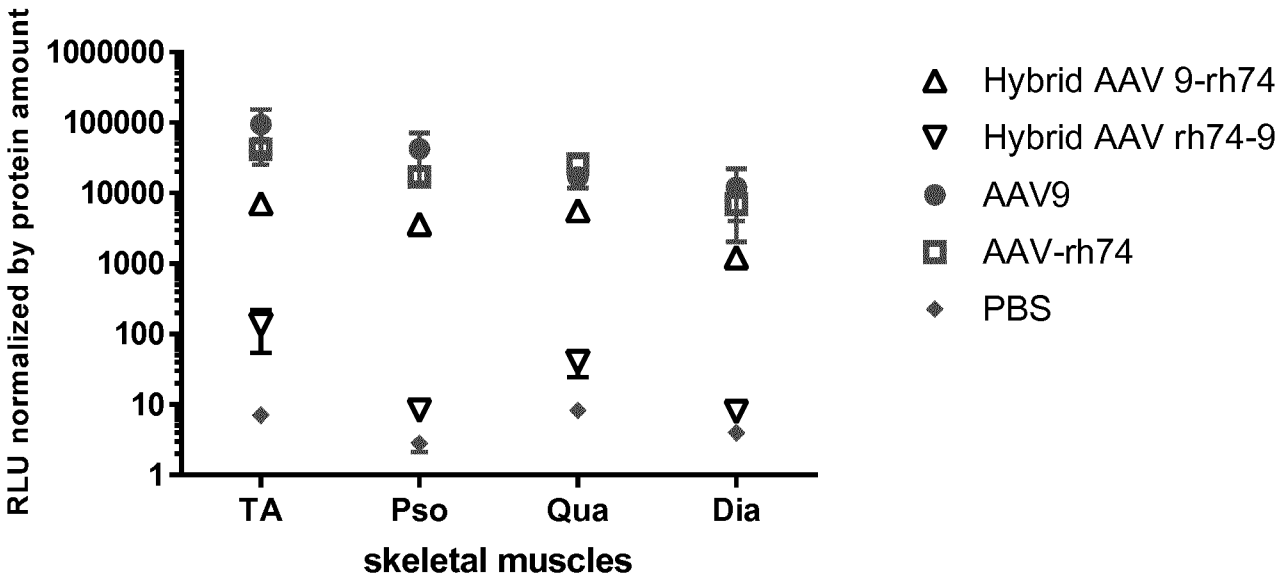
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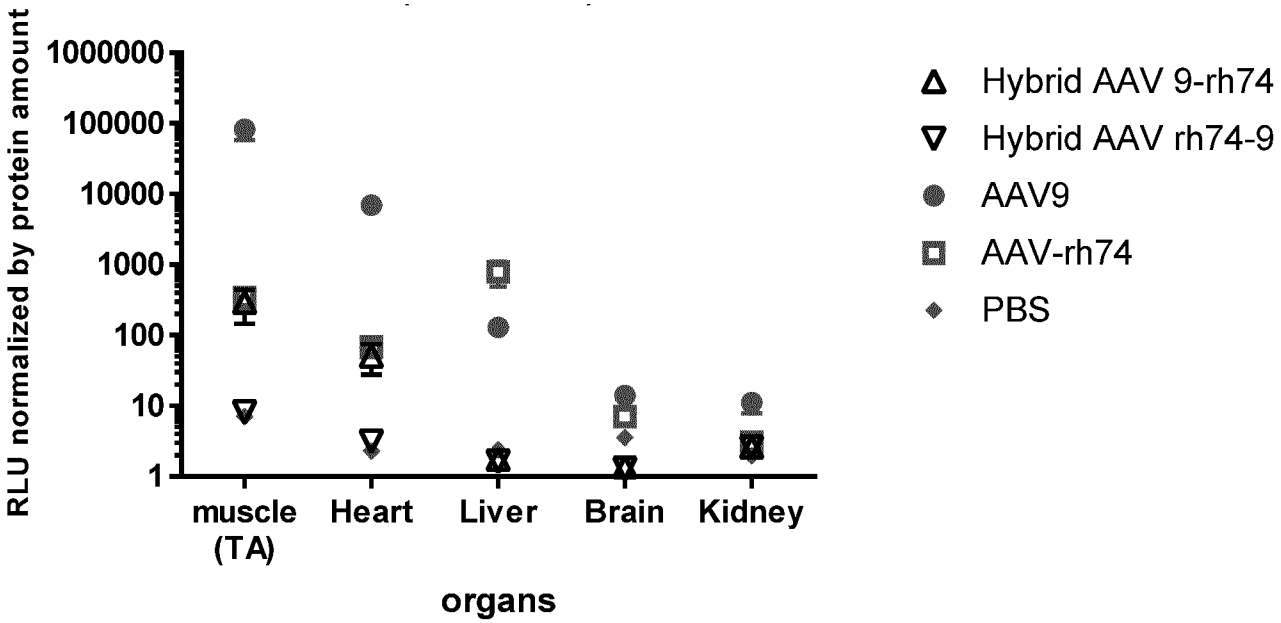
FIGURE 4 (continuation)

C



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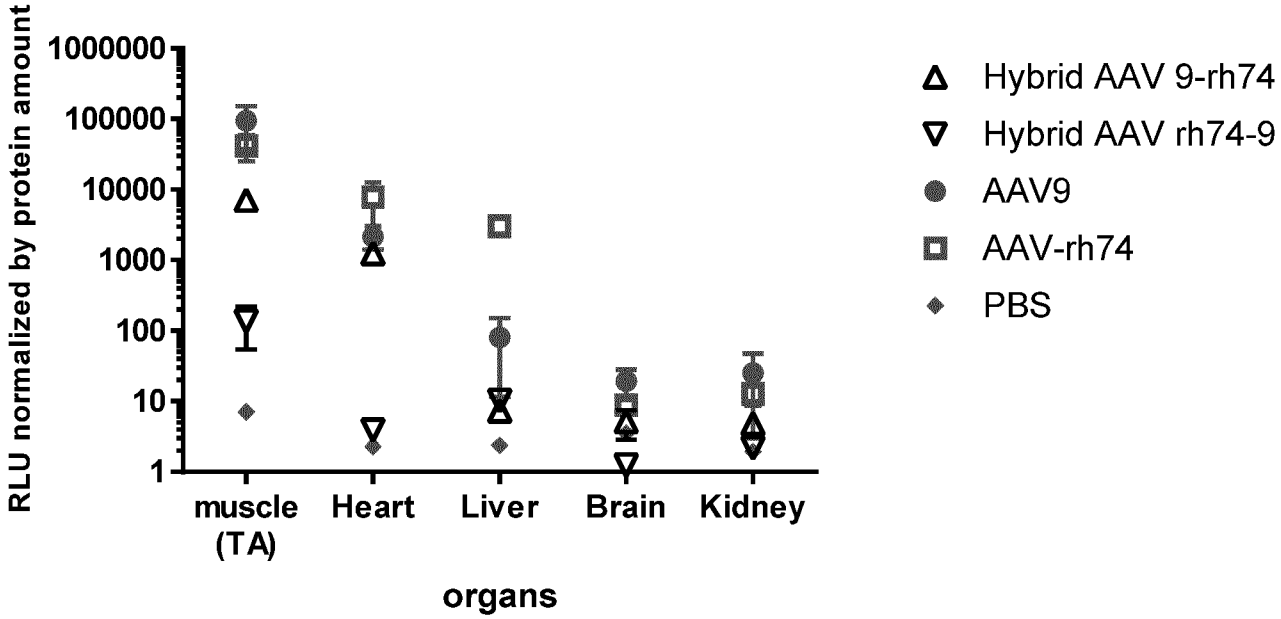

SEQUENCE LISTING

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INSERM
Université d'Evry-val-d'Essone
SORBONNE UNIVERSITE
ASSOCIATION INSTITUT DE MYOLOGIE

<120> HYBRID RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE BETWEEN AAV9 AND AAVrh74 WITH REDUCED LIVER TROPISM

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Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro
        35                  40                  45


Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60


Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80


Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
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Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
145                 150                 155                 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
            180                 185                 190

Ala Ala Pro Ser Gly Val Gly Ser Leu Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
            260                 265                 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
```

```
                325                 330                 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
            340                 345                 350

Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
    370                 375                 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
                405                 410                 415

Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
        435                 440                 445

Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe Ser
    450                 455                 460

Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile Pro
465                 470                 475                 480

Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln Asn
                485                 490                 495

Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu Asn
            500                 505                 510

Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His Lys
        515                 520                 525

Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe Gly
    530                 535                 540
```

```
Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met Ile
545                 550                 555                 560

Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Ser
                565                 570                 575

Tyr Gly Gln Val Ala Thr Asn His Gln Ser Ala Gln Ala Gln Ala Gln
            580                 585                 590

Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp Gln
        595                 600                 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
    610                 615                 620

Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Met
625                 630                 635                 640

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
                645                 650                 655

Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile Thr
            660                 665                 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
        675                 680                 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
    690                 695                 700

Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly Val
705                 710                 715                 720

Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
                725                 730                 735
```

<210> 2
<211> 738
<212> PRT
<213> adeno-associated virus rh74

<400> 2

```
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                  10                  15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
            20                  25                  30

Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Ser Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145                 150                 155                 160

Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
                165                 170                 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
            180                 185                 190

Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
        195                 200                 205
```

```
Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210                 215                 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225                 230                 235                 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
            260                 265                 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
        275                 280                 285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
305                 310                 315                 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
                405                 410                 415

Asn Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430
```

```
Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445

Ser Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu
    450                 455                 460

Phe Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp
465                 470                 475                 480

Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser
                485                 490                 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His
            500                 505                 510

Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr
        515                 520                 525

His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met
    530                 535                 540

Phe Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val
545                 550                 555                 560

Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
                565                 570                 575

Glu Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gln Gln Asn Ala Ala
            580                 585                 590

Pro Ile Val Gly Ala Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
        595                 600                 605

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
    610                 615                 620

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
625                 630                 635                 640
```

```
Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val
                645                 650                 655

Pro Ala Asp Pro Pro Thr Thr Phe Asn Gln Ala Lys Leu Ala Ser Phe
            660                 665                 670

Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu
        675                 680                 685

Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr
    690                 695                 700

Ser Asn Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu
705                 710                 715                 720

Gly Thr Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
                725                 730                 735

Asn Leu
```

<210> 3
<211> 737
<212> PRT
<213> artificial sequence

<220>
<223> synthetic polypeptide AAV9-rh74 hybrid capsid

<400> 3

```
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Gln Pro
            20                  25                  30

Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro
        35                  40                  45

Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60
```

```
Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
145                 150                 155                 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
            180                 185                 190

Ala Ala Pro Ser Gly Val Gly Ser Leu Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
            260                 265                 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285
```

```
Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
                325                 330                 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
            340                 345                 350

Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
    370                 375                 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
                405                 410                 415

Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
        435                 440                 445

Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu Phe
    450                 455                 460

Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp Leu
465                 470                 475                 480

Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser Gln
                485                 490                 495
```

```
Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu
            500                 505                 510

Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr His
        515                 520                 525

Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met Phe
    530                 535                 540

Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val Met
545                 550                 555                 560

Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu
                565                 570                 575

Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gln Gln Asn Ala Ala Pro
            580                 585                 590

Ile Val Gly Ala Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp
        595                 600                 605

Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
    610                 615                 620

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625                 630                 635                 640

Met Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
                645                 650                 655

Ala Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile
            660                 665                 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
        675                 680                 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
    690                 695                 700

Asn Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly
705                 710                 715                 720
```

Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
725 730 735

Leu

<210> 4
<211> 737
<212> PRT
<213> artificial sequence

<220>
<223> synthetic polypeptide AAVrh74-9 hybrid capsid

<400> 4

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Ser Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

```
Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145                 150                 155                 160

Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
                165                 170                 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
            180                 185                 190

Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
        195                 200                 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210                 215                 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225                 230                 235                 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
            260                 265                 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
        275                 280                 285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
305                 310                 315                 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350
```

```
Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
                405                 410                 415

Asn Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445

Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe
    450                 455                 460

Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile
465                 470                 475                 480

Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln
                485                 490                 495

Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu
            500                 505                 510

Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His
        515                 520                 525

Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe
    530                 535                 540

Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met
545                 550                 555                 560

Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu
                565                 570                 575
```

```
Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser Ala Gln Ala Gln Ala
            580                 585                 590

Gln Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp
        595                 600                 605

Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
    610                 615                 620

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625                 630                 635                 640

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
                645                 650                 655

Ala Asp Pro Pro Thr Thr Phe Asn Gln Ala Lys Leu Ala Ser Phe Ile
            660                 665                 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
        675                 680                 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
    690                 695                 700

Asn Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu Gly
705                 710                 715                 720

Thr Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
                725                 730                 735

Leu
```

<210> 5
<211> 2214
<212> DNA
<213> artificial sequence

<220>
<223> synthetic polynucleotide encoding AAV9-rh74 hybrid capsid

<220>
<221> CDS
<222> (1)..(2211)

<400> 5

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atg gct gcc gat ggt tat ctt cca gat tgg ctc gag gac aac ctt agt         48
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                  10                  15

gaa gga att cgc gag tgg tgg gct ttg aaa cct gga gcc cct caa ccc         96
Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Gln Pro
            20                  25                  30

aag gca aat caa caa cat caa gac aac gct cga ggt ctt gtg ctt ccg        144
Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro
        35                  40                  45

ggt tac aaa tac ctt gga ccc ggc aac gga ctc gac aag ggg gag ccg        192
Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

gtc aac gca gca gac gcg gcg gcc ctc gag cac gac aag gcc tac gac        240
Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

cag cag ctc aag gcc gga gac aac ccg tac ctc aag tac aac cac gcc        288
Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
                85                  90                  95

gac gcc gag ttc cag gag cgg ctc aaa gaa gat acg tct ttt ggg ggc        336
Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

aac ctc ggg cga gca gtc ttc cag gcc aaa aag agg ctt ctt gaa cct        384
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
        115                 120                 125

ctt ggt ctg gtt gag gaa gcg gct aag acg gct cct gga aag aag agg        432
Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

cct gta gag cag tct cct cag gaa ccg gac tcc tcc gcg ggt att ggc        480
Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
145                 150                 155                 160

aaa tcg ggt gca cag ccc gct aaa aag aga ctc aat ttc ggt cag act        528
Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

ggc gac aca gag tca gtc cca gac cct caa cca atc gga gaa cct ccc        576
Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
            180                 185                 190
```

```
gca gcc ccc tca ggt gtg gga tct ctt aca atg gct tca ggt ggt ggc        624
Ala Ala Pro Ser Gly Val Gly Ser Leu Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

gca cca gtg gca gac aat aac gaa ggt gcc gat gga gtg ggt agt tcc        672
Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
    210                 215                 220

tcg gga aat tgg cat tgc gat tcc caa tgg ctg ggg gac aga gtc atc        720
Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

acc acc agc acc cga acc tgg gcc ctg ccc acc tac aac aat cac ctc        768
Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

tac aag caa atc tcc aac agc aca tct gga gga tct tca aat gac aac        816
Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
            260                 265                 270

gcc tac ttc ggc tac agc acc ccc tgg ggg tat ttt gac ttc aac aga        864
Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285

ttc cac tgc cac ttc tca cca cgt gac tgg cag cga ctc atc aac aac        912
Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

aac tgg gga ttc cgg cct aag cga ctc aac ttc aag ctc ttc aac att        960
Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

cag gtc aaa gag gtt acg gac aac aat gga gtc aag acc atc gcc aat       1008
Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
                325                 330                 335

aac ctt acc agc acg gtc cag gtc ttc acg gac tca gac tat cag ctc       1056
Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
            340                 345                 350

ccg tac gtg ctc ggg tcg gct cac gag ggc tgc ctc ccg ccg ttc cca       1104
Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

gcg gac gtt ttc atg att cct cag tac ggg tat ctg acg ctt aat gat       1152
Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
    370                 375                 380

gga agc cag gcc gtg ggt cgt tcg tcc ttt tac tgc ctg gaa tat ttc       1200
Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

ccg tcg caa atg cta aga acg ggt aac aac ttc cag ttc agc tac gag       1248
Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
```

```
                405                 410                 415

ttt gag aac gta cct ttc cat agc agc tac gct cac agc caa agc ctg       1296
Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

gac cga cta atg aat cca ctc atc gac caa tac ttg tac tat ctc tca       1344
Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
        435                 440                 445

cgg act caa agc acg ggc ggt act gca gga act cag cag ttg cta ttt       1392
Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu Phe
    450                 455                 460

tct cag gcc ggg cct aac aac atg tcg gct cag gcc aag aac tgg cta       1440
Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp Leu
465                 470                 475                 480

ccc ggt ccc tgc tac cgg cag caa cgc gtc tcc acg aca ctg tcg cag       1488
Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser Gln
                485                 490                 495

aac aac aac agc aac ttt gcc tgg acg ggt gcc acc aag tat cat ctg       1536
Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu
            500                 505                 510

aat ggc aga gac tct ctg gtg aat cct ggc gtt gcc atg gct acc cac       1584
Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr His
        515                 520                 525

aag gac gac gaa gag cga ttt ttt cca tcc agc gga gtc tta atg ttt       1632
Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met Phe
    530                 535                 540

ggg aaa cag gga gct gga aaa gac aac gtg gac tat agc agc gtg atg       1680
Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val Met
545                 550                 555                 560

cta acc agc gag gaa gaa ata aag acc acc aac cca gtg gcc aca gaa       1728
Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu
                565                 570                 575

cag tac ggc gtg gtg gcc gat aac ctg caa cag caa aac gcc gct cct       1776
Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gln Gln Asn Ala Ala Pro
            580                 585                 590

att gta ggg gcc gtc aat agt caa gga gcc tta cct ggc atg gtg tgg       1824
Ile Val Gly Ala Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp
        595                 600                 605

cag aac aga gat gtg tac ctg caa gga ccc att tgg gcc aaa att cct       1872
Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
    610                 615                 620
```

```
cac acg gac ggc aac ttt cac cct tct ccg ctg atg gga ggg ttt gga       1920
His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625                 630                 635                 640

atg aag cac ccg cct cct cag atc ctc atc aaa aac aca cct gta cct       1968
Met Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
                645                 650                 655

gcg gat cct cca acg gcc ttc aac aag gac aag ctg aac tct ttc atc       2016
Ala Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile
            660                 665                 670

acc cag tat tct act ggc caa gtc agc gtg gag atc gag tgg gag ctg       2064
Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
        675                 680                 685

cag aag gaa aac agc aag cgc tgg aac ccg gag atc cag tac act tcc       2112
Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
    690                 695                 700

aac tat tac aag tct aat aat gtt gaa ttt gct gtt aat act gaa ggt       2160
Asn Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly
705                 710                 715                 720

gta tat agt gaa ccc cgc ccc att ggc acc aga tac ctg act cgt aat       2208
Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
                725                 730                 735

ctg taa                                                              2214
Leu
```

<210> 6
<211> 737
<212> PRT
<213> artificial sequence

<220>
<223> Synthetic Construct

<400> 6

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Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1               5                   10                  15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Gln Pro
            20                  25                  30

Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro
        35                  40                  45
```

```
Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
    50                  55                  60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65                  70                  75                  80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
                85                  90                  95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
        115                 120                 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
145                 150                 155                 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
                165                 170                 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
            180                 185                 190

Ala Ala Pro Ser Gly Val Gly Ser Leu Thr Met Ala Ser Gly Gly Gly
        195                 200                 205

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
    210                 215                 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225                 230                 235                 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
                245                 250                 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
```

```
            260                 265                 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
        275                 280                 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
    290                 295                 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305                 310                 315                 320

Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
                325                 330                 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
            340                 345                 350

Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
        355                 360                 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
    370                 375                 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385                 390                 395                 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
                405                 410                 415

Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
            420                 425                 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
        435                 440                 445

Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu Phe
    450                 455                 460

Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp Leu
465                 470                 475                 480
```

```
Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser Gln
                485                 490                 495

Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His Leu
            500                 505                 510

Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr His
        515                 520                 525

Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met Phe
    530                 535                 540

Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val Met
545                 550                 555                 560

Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu
                565                 570                 575

Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gln Gln Asn Ala Ala Pro
            580                 585                 590

Ile Val Gly Ala Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val Trp
        595                 600                 605

Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
    610                 615                 620

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625                 630                 635                 640

Met Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
                645                 650                 655

Ala Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile
            660                 665                 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
        675                 680                 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
```

690 695 700

Asn Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly
705 710 715 720

Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
725 730 735

Leu

<210> 7
<211> 2214
<212> DNA
<213> artificial sequence

<220>
<223> synthetic polynucleotide encoding AAVrh74-9 hybrid capsid

<220>
<221> CDS
<222> (1)..(2211)

<400> 7
atg gct gcc gat ggt tat ctt cca gat tgg ctc gag gac aac ctc tct 48
Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

gag ggc att cgc gag tgg tgg gac ctg aaa cct gga gcc ccg aaa ccc 96
Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

aaa gcc aac cag caa aag cag gac aac ggc cgg ggt ctg gtg ctt cct 144
Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
35 40 45

ggc tac aag tac ctc gga ccc ttc aac gga ctc gac aag ggg gag ccc 192
Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

gtc aac gcg gcg gac gca gcg gcc ctc gag cac gac aag gcc tac gac 240
Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

cag cag ctc caa gcg ggt gac aat ccg tac ctg cgg tat aat cac gcc 288
Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

gac gcc gag ttt cag gag cgt ctg caa gaa gat acg tct ttt ggg ggc 336

```
Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
            100                 105                 110

aac ctc ggg cgc gca gtc ttc cag gcc aaa aag cgg gtt ctc gaa cct        384
Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
        115                 120                 125

ctg ggc ctg gtt gaa tcg ccg gtt aag acg gct cct gga aag aag aga        432
Leu Gly Leu Val Glu Ser Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
    130                 135                 140

ccg gta gag cca tca ccc cag cgc tct cca gac tcc tct acg ggc atc        480
Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145                 150                 155                 160

ggc aag aaa ggc cag cag ccc gca aaa aag aga ctc aat ttt ggg cag        528
Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
                165                 170                 175

act ggc gac tca gag tca gtc ccc gac cct caa cca atc gga gaa cca        576
Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
            180                 185                 190

cca gca ggc ccc tct ggt ctg gga tct ggt aca atg gct gca ggc ggt        624
Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
        195                 200                 205

ggc gct cca atg gca gac aat aac gaa ggc gcc gac gga gtg ggt agt        672
Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210                 215                 220

tcc tca gga aat tgg cat tgc gat tcc aca tgg ctg ggc gac aga gtc        720
Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225                 230                 235                 240

atc acc acc agc acc cgc acc tgg gcc ctg ccc acc tac aac aac cac        768
Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255

ctc tac aag caa atc tcc aac ggg acc tcg gga gga agc acc aac gac        816
Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
            260                 265                 270

aac acc tac ttc ggc tac agc acc ccc tgg ggg tat ttt gac ttc aac        864
Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
        275                 280                 285

aga ttc cac tgc cac ttt tca cca cgt gac tgg cag cga ctc atc aac        912
Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300

aac aac tgg gga ttc cgg ccc aag agg ctc aac ttc aag ctc ttc aac        960
Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
305                 310                 315                 320
```

```
atc caa gtc aag gag gtc acg cag aat gaa ggc acc aag acc atc gcc       1008
Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335

aat aac ctt acc agc acg att cag gtc ttt acg gac tcg gaa tac cag       1056
Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350

ctc ccg tac gtg ctc ggc tcg gcg cac cag ggc tgc ctg cct ccg ttc       1104
Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365

ccg gcg gac gtc ttc atg att cct cag tac ggg tac ctg act ctg aac       1152
Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380

aat ggc agt cag gct gtg ggc cgg tcg tcc ttc tac tgc ctg gag tac       1200
Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400

ttt cct tct caa atg ctg aga acg ggc aac aac ttt gaa ttc agc tac       1248
Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
                405                 410                 415

aac ttc gag gac gtg ccc ttc cac agc agc tac gcg cac agc cag agc       1296
Asn Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430

ctg gac cgg ctg atg aac cct ctc atc gac cag tac ttg tac tac ctg       1344
Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445

tcc aag act att aac ggt tct gga cag aat caa caa acg cta aaa ttc       1392
Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe
    450                 455                 460

agt gtg gcc gga ccc agc aac atg gct gtc cag gga aga aac tac ata       1440
Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile
465                 470                 475                 480

cct gga ccc agc tac cga caa caa cgt gtc tca acc act gtg act caa       1488
Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln
                485                 490                 495

aac aac aac agc gaa ttt gct tgg cct gga gct tct tct tgg gct ctc       1536
Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu
            500                 505                 510

aat gga cgt aat agc ttg atg aat cct gga cct gct atg gcc agc cac       1584
Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His
        515                 520                 525

aaa gaa gga gag gac cgt ttc ttt cct ttg tct gga tct tta att ttt       1632
```

```
Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe
    530                 535                 540

ggc aaa caa gga act gga aga gac aac gtg gat gcg gac aaa gtc atg       1680
Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met
545                 550                 555                 560

ata acc aac gaa gaa gaa att aaa act act aac ccg gta gca acg gag       1728
Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu
                565                 570                 575

tcc tat gga caa gtg gcc aca aac cac cag agt gcc caa gca cag gcg       1776
Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser Ala Gln Ala Gln Ala
            580                 585                 590

cag acc ggc tgg gtt caa aac caa gga ata ctt ccg ggt atg gtt tgg       1824
Gln Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp
        595                 600                 605

cag gac cgg gac gtg tac ctg cag ggt ccc atc tgg gcc aag att cct       1872
Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
    610                 615                 620

cat acg gac ggc aac ttt cat ccc tcg ccg ctg atg gga ggc ttt gga       1920
His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625                 630                 635                 640

ctg aag cat ccg cct cct cag atc ctg att aaa aac aca cct gtt ccc       1968
Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
                645                 650                 655

gcg gat cct ccg acc acc ttc aat cag gcc aag ctg gct tct ttc atc       2016
Ala Asp Pro Pro Thr Thr Phe Asn Gln Ala Lys Leu Ala Ser Phe Ile
            660                 665                 670

acg cag tac agt acc ggc cag gtc agc gtg gag atc gag tgg gag ctg       2064
Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
        675                 680                 685

cag aag gag aac agc aaa cgc tgg aac cca gag att cag tac act tcc       2112
Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
    690                 695                 700

aac tac tac aaa tct aca aat gtg gac ttt gct gtc aat act gag ggt       2160
Asn Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu Gly
705                 710                 715                 720

act tat tcc gag cct cgc ccc att ggc acc cgt tac ctc acc cgt aat       2208
Thr Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
                725                 730                 735

ctg taa                                                              2214
Leu
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<210> 8
<211> 737
<212> PRT
<213> artificial sequence

<220>
<223> Synthetic Construct

<400> 8

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Gln Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Ser Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145 150 155 160

Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
165 170 175

```
Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
            180                 185                 190

Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
        195                 200                 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
    210                 215                 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
225                 230                 235                 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
                245                 250                 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
            260                 265                 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
        275                 280                 285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
    290                 295                 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
305                 310                 315                 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
                325                 330                 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
            340                 345                 350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
        355                 360                 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
    370                 375                 380
```

```
Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
385                 390                 395                 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
                405                 410                 415

Asn Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
            420                 425                 430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
        435                 440                 445

Ser Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe
    450                 455                 460

Ser Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile
465                 470                 475                 480

Pro Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln
                485                 490                 495

Asn Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu
            500                 505                 510

Asn Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His
        515                 520                 525

Lys Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe
    530                 535                 540

Gly Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met
545                 550                 555                 560

Ile Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu
                565                 570                 575

Ser Tyr Gly Gln Val Ala Thr Asn His Gln Ser Ala Gln Ala Gln Ala
            580                 585                 590

Gln Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp
        595                 600                 605
```

Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
610 615 620

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
625 630 635 640

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
645 650 655

Ala Asp Pro Pro Thr Thr Phe Asn Gln Ala Lys Leu Ala Ser Phe Ile
660 665 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
675 680 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
690 695 700

Asn Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Glu Gly
705 710 715 720

Thr Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
725 730 735

Leu

<210> 9
<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> synthetic peptide

<400> 9

Lys Thr Ile Asn Gly Ser
1 5

<210> 10
<211> 7
<212> PRT

<213> artificial sequence

<220>
<223> synthetic peptide

<400> 10

Pro Gly Met Val Trp Gln Asp
1 5

<210> 11
<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> synthetic peptide

<400> 11

Arg Thr Gln Ser Thr Gly
1 5

<210> 12
<211> 7
<212> PRT
<213> artificial sequence

<220>
<223> synthetic peptide

<400> 12

Pro Gly Met Val Trp Gln Asn
1 5