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(54) Titre : SEROTYPE DU VIRUS ADENO-ASSOCIE RECOMBINANT HYBRIDE ENTRE AAV9 ET AAVRH74 POSSEDANT UN TROPISME HEPATIQUE REDUIT

(54) Title: HYBRID RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE BETWEEN AAV9 AND AAVRH74 WITH REDUCED LIVER TROPISM

(57) Abrégé/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.

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(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.

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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

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 (**- Figure *1*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.

In some embodiments, the hybrid AAV capsid protein of the invention further comprises, additional modifications, for example modifications which increase the targeting of skeletal or cardiac muscle tissue by AAV vectors. A non-limiting example is the fusion of Anthopleurin-B to the N-terminus of AAV VP2 capsid protein (Finet *et al.*, Virology, 2018, 513, 43-51). Another modification is the insertion of a peptide into a site exposed on the capsid surface, in particular around position 588 according to the numbering in SEQ ID NO:

1. Non-limiting examples of such peptides are disclosed in Michelfelder et al. (PLoS ONE, 2009, 4, e5122). The insertion site is advantageously from positions 587 to 592 according to the numbering in SEQ ID NO: 1. The insertion of the peptide may or may not cause the deletion of some or all of the residue(s) from the insertion site. The peptide has advantageously a sequence of no more than 20 amino acids which may include fixed sequences of no more than five amino acids at its N- and/or C-terminal ends, such as for example GQSG (SEQ ID NO: 35) and AQAA (SEQ ID NO: 36), respectively at the N- and C-terminal end of the peptide.

In some embodiments, the peptide comprises or consists of a sequence selected from the group consisting of SEQ ID NO: 12 to 34. Preferably, said peptide is flanked by GQSG (SEQ ID NO: 35) and AQAA (SEQ ID NO: 36), respectively at its N- and C-terminal end. The peptide advantageously replaces all the residues from positions 587 to 592 of the AAV capsid protein according to the numbering in SEQ ID NO: 1. The peptide advantageously increases the targeting of cardiac muscle tissue and eventually also of skeletal muscle tissue. In some preferred embodiment, the peptide-modified hybrid AAV capsid protein comprises or consists of a sequence selected from the group consisting of SEQ ID NO: 9 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:9. SEQ ID NO: 9 is derived from the hybrid Cap9-rh74 of SEQ ID NO: 3 by the insertion of the peptide of SEQ ID NO: 12. 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

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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

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

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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: 37 and SEQ ID NO: 38 for AAV9 and SEQ ID NO: 39 and SEQ ID NO: 40 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.

EXAMPLE 3: Peptide-modified hybrid AAV9-rh74 serotype vector

The sequence of the hybrid Cap9-rh74 was modified with a peptide to increase hybrid AAV 9-rh74 serotype vector tropism for muscle tissue. AAV capsid modification was performed according to Kienle EC (Dissertation for the degree of Doctor of natural Sciences, Combined Faculties for the Natural Sciences and for Mathematics of the Ruperto-Carola University of Heidelberg, Germany, 2014) using a peptide as disclosed in Michelfelder et al. (PLoS ONE, 2009, 4, e5122). Briefly, the hexapeptide QQNAAP (SEQ ID NO: 41) present in the VP1 of the hybrid Cap9-rh74 (positions 587 to 592 of SEQ ID NO: 3) is mutated to the octapeptide GQSGAQAA (SEQ ID NO: 42) and peptide P1 (RGDLGLS; SEQ ID NO: 12) is inserted between glycine at position 4 and alanine at position 5. The hybrid Cap9-rh74 modified with peptide P1 has the amino acid sequence SEQ ID NO: 9 and the corresponding coding sequence is SEQ ID NO: 10. Vectors are produced by triple transfection in HEK293 cells grown in suspension and purified by affinity chromatography as described in example 1. Vectors are injected in mice, one month after the injection mice are sacrificed and tissues collected to evaluate the biodistribution as described in example 2. The expected effect of this modification is the increase of the quantity of vector in muscle tissues.

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.

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.

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 SEQ ID NO: 3 and SEQ ID NO: 4 and the sequences having at least 85%, 90%, 95%, 97%, 98% or 99% identity with said sequences, preferably which comprises a sequence selected from the

group consisting of the sequences 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.

6. The recombinant hybrid AAV capsid protein according to any one of claims 1 to 5, which comprises the insertion of a peptide which increases the targeting of skeletal or cardiac muscle tissue by AAV vectors.

7. The recombinant hybrid AAV capsid protein according to claim 6, wherein said peptide comprises a sequence selected from the group consisting of SEQ ID NO: 12 to 34.

8. The recombinant hybrid AAV capsid protein according to claim 6 or 7, which comprises a sequence selected from the group consisting of SEQ ID NO: 9 and the sequences having at least 85%, 90%, 95%, 97%, 98% or 99% identity with said sequence.

9. The recombinant hybrid AAV capsid protein according to any one of claims 1 to 8, which is a hybrid VP1, VP2 or VP3 protein.

10. 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 6, 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 6.

11. A polynucleotide encoding the recombinant hybrid AAV capsid protein according to any one of claims 1 to 9 or the recombinant chimeric AAV capsid protein according to claim 10, in expressible form, and eventually further encoding AAV Replicase protein in expressible form.

12. A recombinant plasmid comprising the polynucleotide of claim 11.

13. 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 9, and/or the recombinant chimeric AAV capsid protein according to claim 10, and eventually also at least one AAV capsid protein from natural or artificial AAV serotype other than AAV9 and AAVrh74.

14. The AAV vector particle according to claim 13, 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.

15. A pharmaceutical composition comprising a therapeutically effective amount of AAV vector particles according to claim 13 or claim 14.

16. The pharmaceutical composition of claim 15, which is for use as a medicament in gene therapy, preferably for treating genetic diseases, cancer or auto-immune diseases affecting muscle tissues.

17. The pharmaceutical composition for the use according to claim 16, which 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.

18. The pharmaceutical composition for the use according to claim 17, wherein the target gene is selected from the group comprising: *DMD*, *BMD*, *CAPN3*, *DUX4*, *FRG1*, *SMCHD1* and TTN genes.

FIGURE 1

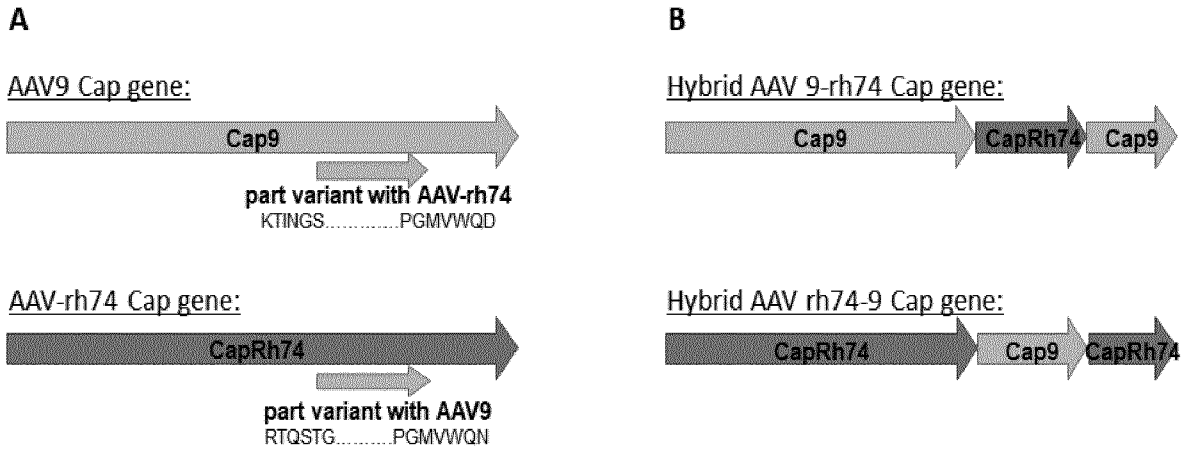


FIGURE 2

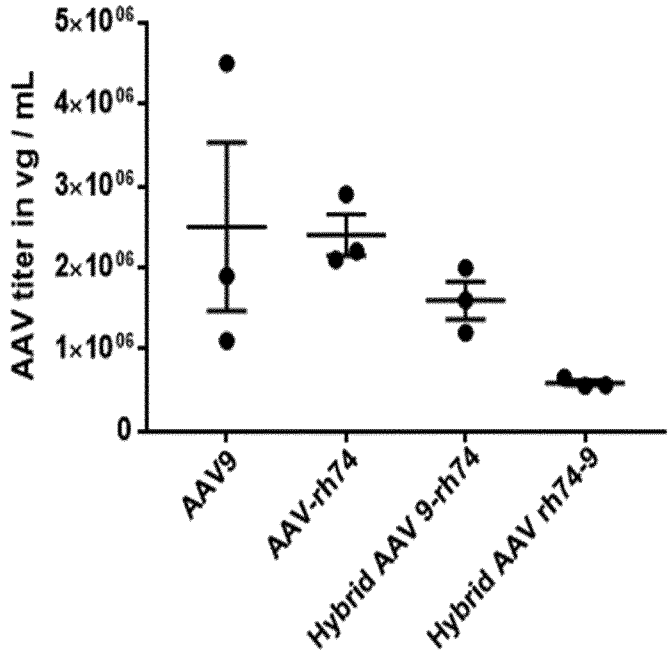


FIGURE 3

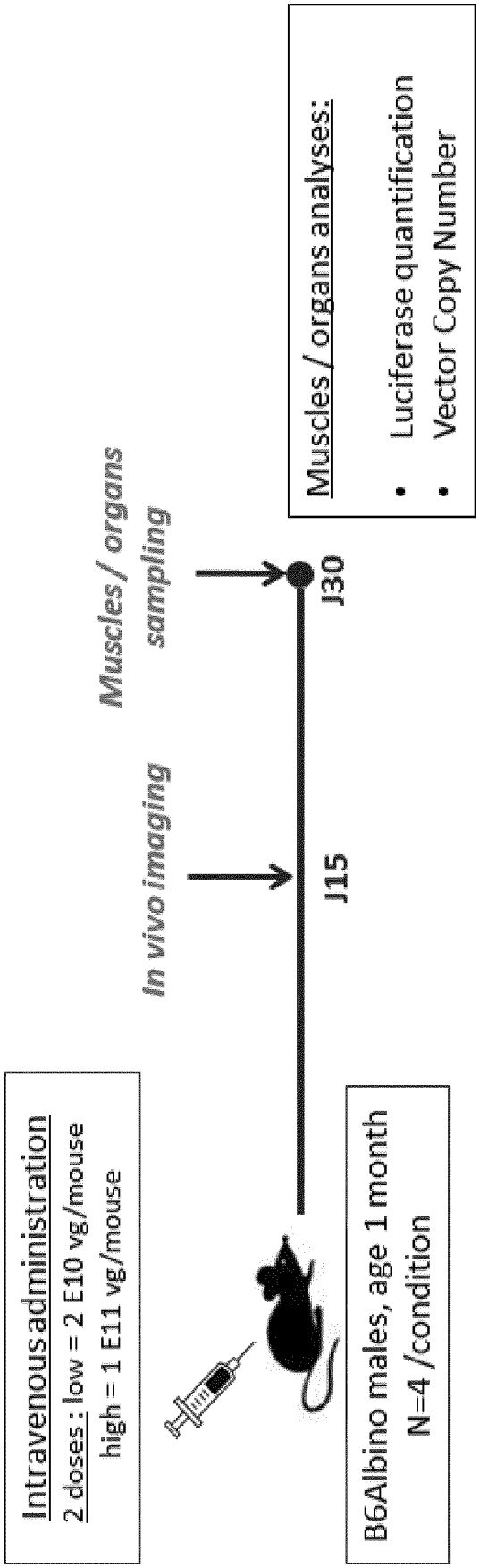
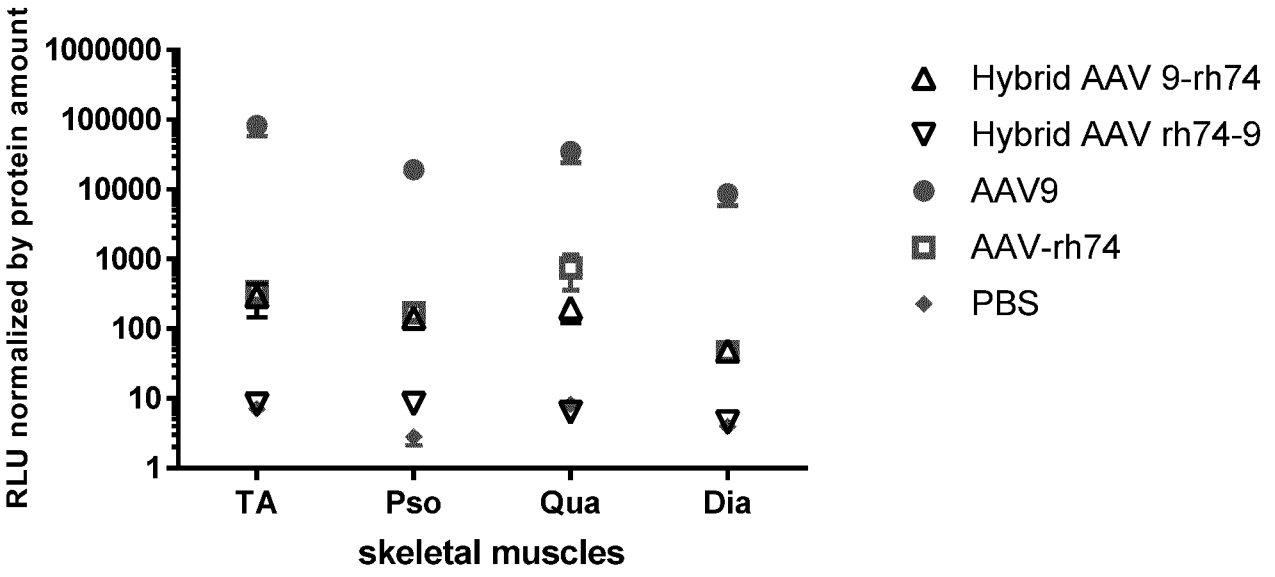

Intravenous administration
2 doses : low = 2 E10 vg/mouse
high = 1 E11 vg/mouse
B6Albino males, age 1 month
N=4 /condition
In vivo imaging
J15
Muscles / organs sampling
J30
Muscles / organs analyses:
Luciferase quantification
Vector Copy Number

FIGURE 4

A


RLU normalized by protein amount
1000000
100000
10000
1000
100
10
1
TA
Pso
Qua
Dia
skeletal muscles
Hybrid AAV 9-rh74
Hybrid AAV rh74-9
AAV9
AAV-rh74
PBS

B

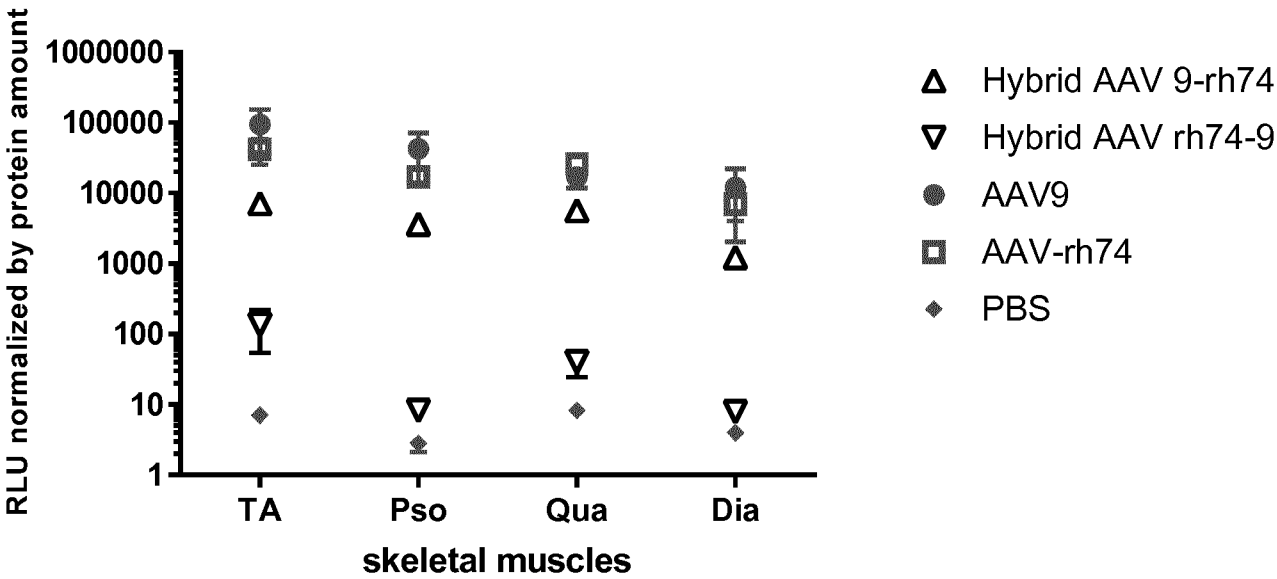
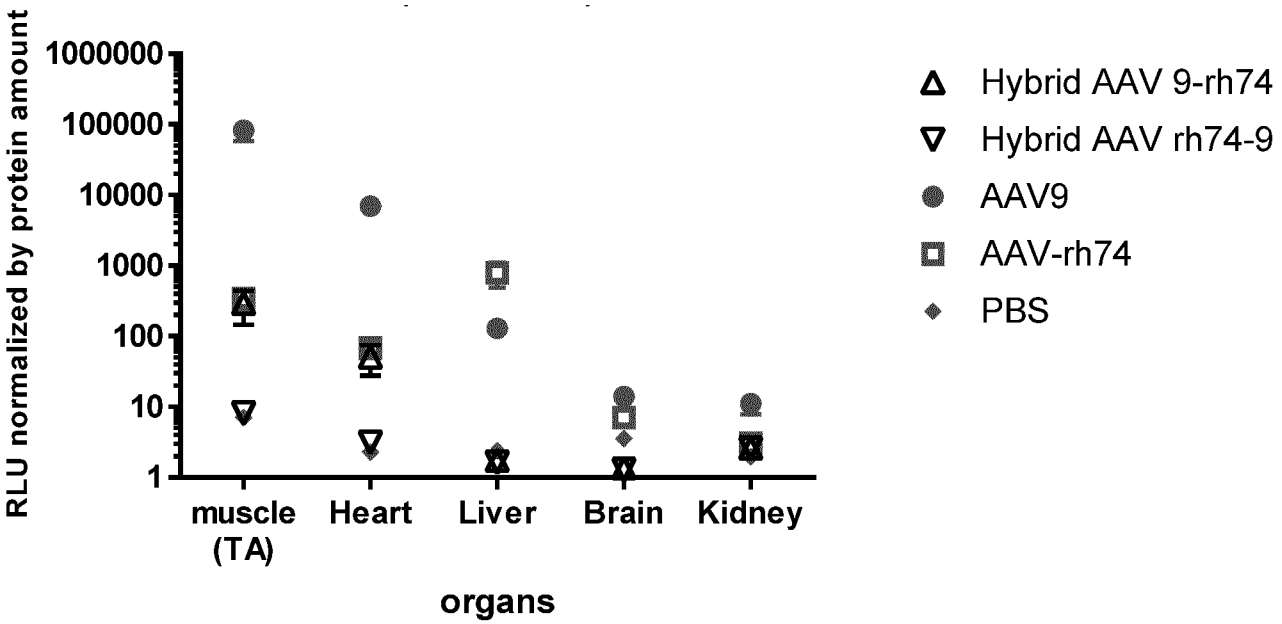

RLU normalized by protein amount
1000000
100000
10000
1000
100
10
1
TA
Pso
Qua
Dia
skeletal muscles
Hybrid AAV 9-rh74
Hybrid AAV rh74-9
AAV9
AAV-rh74
PBS

FIGURE 4 (continuation)

C



D