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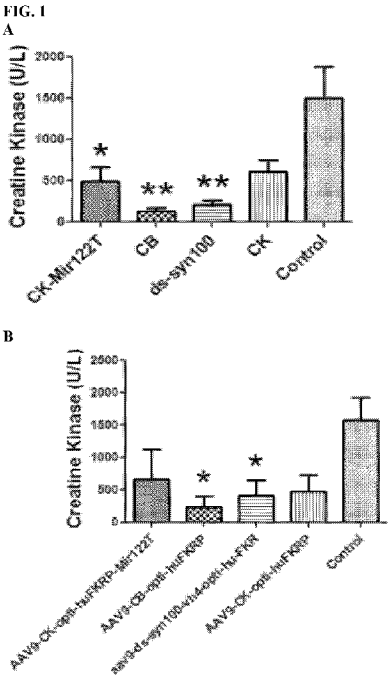
(57) Abstract: The invention relates to synthetic polynucleotides encoding fukutin related protein (FKRP). The invention further relates to nucleic acid constructs comprising the synthetic polynucleotides and methods of using these synthetic polynucleotides to treat dystroglycanopathy disorders.



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OPTIMIZED FUKUTIN-RELATED PROTEINS (FKRP) AND METHODS OF USE

STATEMENT OF PRIORITY

This application claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 63/133,615, filed on January 4, 2021, the entire contents of which are incorporated by reference herein.

STATEMENT OF FEDERAL SUPPORT

This invention was made with government support under Grant Number 1RO1 AR45967 awarded by the National Institutes of Health. The government has certain rights in the invention.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5470-874WO_ST25.txt, 13,114 bytes in size, generated on December 30, 2021, and filed via EFS-Web, is provided in lieu of a paper copy. This Sequence Listing is hereby incorporated herein by reference into the specification for its disclosures.

FIELD OF THE INVENTION

The invention relates to synthetic polynucleotides encoding fukutin related protein (FKRP). The invention further relates to nucleic acid constructs comprising the synthetic polynucleotides and methods of using these polynucleotides to treat dystroglycanopathy disorders (e.g., that involve a reduction in glycosylation of alpha-dystroglycan (α-DG).

BACKGROUND OF THE INVENTION

Mutations in fukutin-related protein (FKRP) gene cause a wide spectrum of disease phenotypes, including the mild limb-girdle muscular dystrophy 2I (LGMD2I), the severe Walker-Warburg syndrome, and muscle-eye-brain disease. Presently, there is no cure for FKRP-related disorders. Adeno-associated viral (AAV)-mediated gene therapy has been demonstrated effective and robust in FKRP-mutant mouse models and offers great hope for human patients. However, there are certain limitations in the therapeutic effects of the known gene therapies

The present invention overcomes previous shortcomings in the art by providing improved compositions and methods for treating diseases associated with FKRP mutations.

SUMMARY OF THE INVENTION

The present invention is based in part on the surprising finding that double stranded or self-complementary AAV vectors and/or a novel CpG depleted polynucleotide encoding a fukutin-related protein (FKRP) provide improved therapeutic effects over what has previously been known. Accordingly, in one aspect, the invention provides a synthetic polynucleotide encoding a human FKRP, wherein the synthetic polynucleotide comprises the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**.

In another aspect, a synthetic polynucleotide encoding a human fukutin-related protein (FKRP) is provided, wherein the synthetic polynucleotide comprises the nucleotide sequence of **SEQ ID NO:6** and/or a nucleotide sequence having at least 80% identity to the nucleotide sequence of **SEQ ID NO:6**.

In a further aspect, the invention provides a synthetic polynucleotide encoding a human fukutin-related protein (FKRP), wherein the synthetic polynucleotide comprises the nucleotide sequence of **SEQ ID NO:7** and/or a nucleotide sequence having at least 80% identity to the nucleotide sequence of **SEQ ID NO:7**.

In an additional aspect, the invention provides a transformed cell comprising a synthetic polynucleotide of the invention and/or a vector comprising the synthetic polynucleotide of the invention. In some aspects, the invention provides a transgenic animal comprising the synthetic polynucleotide, the vector, and/or the transformed cell of the invention.

In some aspects, a method of delivering a nucleic acid to a cell is provided, the method comprising delivering to the cell a synthetic polynucleotide of the invention, and/or a vector and/or a transformed cell comprising the same. In some aspects, the invention provides a method of delivering a nucleic acid to a subject, the method comprising delivering to the subject a synthetic polynucleotide of the invention, and/or a vector and/or a transformed cell comprising the same. In some embodiments, the synthetic polynucleotide of the invention, and/or vector and/or transformed cell may be delivered to the subject in a therapeutically effective amount.

In further aspects, the invention provides a method of treating a dystroglycanopathy in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of a synthetic polynucleotide of the invention, and/or a vector and/or a transformed cell comprising the same, thereby treating dystroglycanopathy in the subject. In some aspects of the invention, the dystroglycanopathy comprises a mutation in the nucleic acid encoding FKRP and/or a deficiency in glycosylation of alpha-dystroglycan (α-DG), or any combination thereof.

In some aspects of the invention, the dystroglycanopathy is limb girdle muscular dystrophy 2I, congenital muscular dystrophy, Walker-Warburg syndrome, muscle-eye-brain disease, or any combination thereof.

These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Short-term and long-term therapeutic effects in reducing serum CK level mediated by AAV9-opti-hu-FKRP vectors driven by different promoters. Panel A shows short term effects (3 months post injection); Panel B shows long term effects (8 months post injection); Panel C shows long term effects (15 months post injection). * $p< 0.05$; ** $p < 0.01$ as compared to the control group. In the long-term effects group, there was no statistical difference observed because of the limited animal numbers.

FIG. 2. Muscle functional analysis. Left panel. treadmill running distance test. Right panel. Grip force measurement. The muscle force (g) was normalized by body weight (g). * $p< 0.05$; ** $p< 0.01$ as compared to the control group.

FIG. 3. Protein expression of varied hu-FKRP AAV plasmids driven by different promoters revealed via immunofluorescent staining after transfection in 293 cells.

FIG. 4. Serum IL-17A level. There was no statistical difference among groups because of the variances of the animals.

FIG. 5. Constructions of ds-AAV plasmids containing FKRP gene. ITR: inverted terminal repeat; D^{mu}: D-sequence mutation; syn100: synthetic muscle-specific promoter.

FIG. 6. Therapeutic effects mediated by different vectors in reducing serum CK level. Panel A shows male 6weeks post injection; Panel B shows mail 12 weeks post injection; and Panel C shows male 7.5 months post injection. **, $p < 0.01$ as compared to the untreated group. Overall, the CpG(-) performed better than the CpC(+) vectors.

DETAILED DESCRIPTION OF THE INVENTION

The present invention now will be described hereinafter with reference to the accompanying drawings and examples, in which embodiments of the invention are shown. This description is not intended to be a detailed catalog of all the different ways in which the invention may be implemented, or all the features that may be added to the instant invention. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be

deleted from that embodiment. Thus, the invention contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant invention. Hence, the following descriptions are intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention.

All publications, patent applications, patents and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

The term "about," as used herein when referring to a measurable value such as an amount or concentration and the like, is meant to encompass variations of ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified value as well as the specified value. For example, "about X" where X is the measurable value, is meant to include X as well as variations of ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of X. A range provided herein for a measurable value may include any other range and/or individual value therein.

As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y" and phrases such as "from about X to Y" mean "from about X to about Y."

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if the range 10 to15 is disclosed, then 11, 12, 13, and 14 are also disclosed.

The term "comprise," "comprises" and "comprising" as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising."

As used herein, the terms "increase," "increasing," "enhance," "enhancing," "improve" and "improving" (and grammatical variations thereof) as used herein refers to an increase in the specified parameter of at least about 1.25-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 8-fold, 10-fold, twelve-fold, or even fifteen-fold or an increase of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 225%, 250%, 275%, 300%, 400%, 500% or more.

As used herein, the terms "reduce," "reduced," "reducing," "reduction," "diminish," and "decrease" (and grammatical variations thereof), describe, for example, a decrease of at least about 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% as compared to a control. In particular embodiments, the reduction can result in no or essentially no (*i.e.*, an insignificant amount, *e.g.*, less than about 10% or even 5%) detectable activity or amount.

The above terms are relative to a reference or control. For example, in a method of this invention for reducing serum creatine kinase levels in a subject comprising delivering to the subject a synthetic polynucleotide of the invention, the reduction is relative to the amount of

serum creatine kinase in a subject (e.g., a control subject) in the absence of delivery of the synthetic polynucleotide to that control subject.

A "treatment effective" amount or "therapeutically effective" amount as used herein is an amount that is sufficient to provide some improvement or benefit to the subject. Alternatively stated, a "treatment effective" amount is an amount that will provide some alleviation, mitigation, decrease or stabilization in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

Determination of a therapeutically effective amount, as well as other factors related to effective administration of a compound of the present invention to a subject of this invention, including dosage forms, routes of administration, and frequency of dosing, may depend upon the particulars of the condition that is encountered, including the subject and condition being treated or addressed, the severity of the condition in a particular subject, the particular compound being employed, the particular route of administration being employed, the frequency of dosing, and the particular formulation being employed. Determination of a therapeutically effective treatment regimen for a subject of this invention is within the level of ordinary skill in the medical or veterinarian arts. In clinical use, an effective amount may be the amount that is recommended by the U.S. Food and Drug Administration, or an equivalent foreign agency. The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the subject being treated and the particular mode of administration.

In some embodiments, an "treatment effective" amount or "therapeutically effective" amount can refer to an amount of a composition of this invention (*e.g.,* the nucleotide sequence of **SEQ ID NO:1, SEQ ID NO:6,** and/or **SEQ ID NO:7**; a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**; a nucleotide sequence having at least 80% identity to **SEQ ID NO:6** or **SEQ ID NO:7**; a nucleotide sequence encoding a FKRP that is CpG depleted, and/or an expression cassette, vector or transgenic cell comprising one or more of the nucleotide sequence of **SEQ ID NO:1, SEQ ID NO:6,** and/or **SEQ ID NO:7**; a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**; a nucleotide sequence having at least 80% identity to **SEQ ID NO:6** or **SEQ ID NO:7**) that is sufficient to produce a desired effect, which can be a therapeutic effect. The effective amount will vary with the age, general condition of the subject, the severity of the condition being treated, the particular agent administered, the duration of the treatment, the nature of any concurrent treatment, the pharmaceutically acceptable carrier used, and like factors within the knowledge and expertise of those skilled in

the art. As appropriate, an "effective amount" in any individual case can be determined by one of ordinary skill in the art by reference to the pertinent texts and literature and/or by using routine experimentation. (*See*, for example, Remington, *The Science And Practice of Pharmacy* (20th ed. 2000)).

A "prevention effective" amount as used herein is an amount that is sufficient to prevent and/or delay the onset of a disease, disorder and/or clinical symptoms in a subject and/or to reduce and/or delay the severity of the onset of a disease, disorder and/or clinical symptoms in a subject relative to what would occur in the absence of the methods of the invention. Those skilled in the art will appreciate that the level of prevention need not be complete, as long as some benefit is provided to the subject.

The efficacy of treating a dystroglycanopathy by the methods of the invention can be determined by detecting a clinical improvement as indicated by a change in the subject's symptoms and/or clinical parameters as would be well known to one of skill in the art.

By the terms "treat," "treating," or "treatment," it is intended that the severity of the subject's condition is reduced or at least partially improved or modified and that some alleviation, mitigation or decrease in at least one clinical symptom is achieved.

The terms "prevent," "preventing," and "prevention" (and grammatical variations thereof) refer to a decrease or delay in the extent or severity of a disease, disorder and/or clinical symptom(s) after onset relative to what would occur in the absence of carrying out the methods of the invention prior to the onset of the disease, disorder and/or clinical symptom(s). In terms of dystroglycanopathy, "preventing" refers to the occurrence of an increase in glycosylation of alpha-dystroglycan (α-DG) as compared to the amount of glycosylation of alpha-dystroglycan (α-DG) that occurs in the absence of the therapeutic treatment. Thus, a subject identified to have one or more mutations that are associated with a dystroglycanopathy characterized by reduced glycosylation of α-DG can be administered the synthetic/optimized polynucleotides of this invention to prevent/delay/alleviate onset of said dystroglycanopathy. Such mutations are well known in the art.

"Concurrently administering" or "concurrently administer" as used herein means that the two or more compounds or compositions are administered closely enough in time to produce a combined effect (that is, concurrently may be simultaneously, or it may be two or more events occurring within a short time period before and/or after each other, *e.g.*, sequentially). Simultaneous concurrent administration may be carried out by mixing the compounds prior to administration, or by administering the compounds at the same point in time but at different anatomic sites and/or by using different routes of administration.

"Pharmaceutically acceptable" as used herein means that the compound or composition is suitable for administration to a subject to achieve the treatments described herein, without unduly deleterious side effects in light of the severity of the disease and necessity of the treatment.

As used herein, "nucleic acid," "nucleotide sequence," and "polynucleotide" are used interchangeably and encompass both RNA and DNA, including cDNA, genomic DNA, mRNA, synthetic (*e.g.*, chemically synthesized) DNA or RNA and chimeras of RNA and DNA. The term polynucleotide, nucleotide sequence, or nucleic acid refers to a chain of nucleotides without regard to length of the chain. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be a sense strand or an antisense strand. The nucleic acid can be synthesized using oligonucleotide analogs or derivatives (*e.g.*, inosine or phosphorothioate nucleotides) or produced by cell biology techniques commonly used for vector production. Such oligonucleotides can be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases. The present invention further provides a nucleic acid that is the complement (which can be either a full complement or a partial complement) of a nucleic acid, nucleotide sequence, or polynucleotide of this invention.

An "isolated polynucleotide" is a nucleotide sequence (*e.g.*, an "isolated DNA" or an "isolated RNA") that is not immediately contiguous with nucleotide sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. Thus, in one embodiment, an isolated nucleic acid may include some or all of the 5' non-coding (*e.g.*, promoter) sequences that are immediately contiguous to a coding sequence. The term therefore includes, for example, a recombinant DNA/synthetic polynucleotide that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (*e.g.*, a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment), independent of other sequences. It also includes a recombinant DNA/synthetic polynucleotide that is part of a hybrid nucleic acid encoding an additional polypeptide or peptide sequence. An isolated polynucleotide that includes a gene is not a fragment of a chromosome that includes such gene, but rather includes the coding region and regulatory regions associated with the gene, but no additional genes naturally found on the chromosome.

Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right, unless specifically indicated otherwise. Nucleotides and amino acids are

represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by either the one-letter code, or the three letter code, both in accordance with 37 C.F.R. §1.822 and established usage.

Except as otherwise indicated, standard methods known to those skilled in the art may be used for production of recombinant and synthetic polypeptides, antibodies or antigen-binding fragments thereof, manipulation of nucleic acid sequences, production of transformed cells, the construction of rAAV constructs, modified capsid proteins, packaging vectors expressing the AAV rep and/or cap sequences, and transiently and stably transfected packaging cells. Such techniques are known to those skilled in the art. *See, e.g.*, SAMBROOK *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed. (Cold Spring Harbor, NY, 1989); F. M. AUSUBEL *et al.* CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York).

As used herein, the term "modified," as applied to a polynucleotide or polypeptide sequence, refers to a sequence that differs from a wild-type sequence due to one or more deletions, additions, substitutions, or any combination thereof.

The terms "5' portion" and "3' portion" are relative terms to define a spatial relationship between two or more elements. Thus, for example, a "3' portion" of a polynucleotide indicates a segment of the polynucleotide that is downstream of another segment. The term "3' portion" is not intended to indicate that the segment is necessarily at the 3' end of the polynucleotide, or even that it is necessarily in the 3' half of the polynucleotide, although it may be. Likewise, a "5' portion" of a polynucleotide indicates a segment of the polynucleotide that is upstream of another segment. The term "5' portion" is not intended to indicate that the segment is necessarily at the 5' end of the polynucleotide, or even that it is necessarily in the 5' half of the polynucleotide, although it may be. As used herein, the term "polypeptide" encompasses both peptides and proteins, unless indicated otherwise.

The terms "exogenous" and/or "heterologous" as used herein can include a nucleotide sequence that is not naturally occurring in the nucleic acid construct and/or delivery vector (*e.g.*, virus delivery vector) in which it is contained and can also include a nucleotide sequence that is placed into a non-naturally occurring environment and/or position relative to other nucleotide sequences (*e.g.*, by association with a promoter or coding sequence with which it is not naturally associated). A heterologous or exogenous nucleotide sequence or amino acid sequence of this invention can be any heterologous nucleotide sequence and/or amino acid sequence that has been introduced into a cell and can include a nucleotide sequence and/or amino acid sequence for which an original version is already present in the cell and/or the

heterologous nucleotide sequence or amino acid sequence can be introduced into a cell that does not naturally comprise the same nucleotide sequence and/or amino acid sequence.

The term "sequence identity," as used herein, has the standard meaning in the art. As is known in the art, a number of different programs can be used to identify whether a polynucleotide or polypeptide has sequence identity or similarity to a known sequence. Sequence identity or similarity may be determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman, *Adv. Appl. Math. 2*:482 (1981), by the sequence identity alignment algorithm of Needleman & Wunsch, *J. Mol. Biol. 48*:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Natl. Acad. Sci. USA 85*:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux *et al.*, *Nucl. Acid Res. 12*:387 (1984), preferably using the default settings, or by inspection.

An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol. 35*:351 (1987); the method is similar to that described by Higgins & Sharp, *CABIOS 5*:151 (1989).

Another example of a useful algorithm is the BLAST algorithm, described in Altschul *et al.*, *J. Mol. Biol. 215*:403 (1990) and Karlin *et al.*, *Proc. Natl. Acad. Sci. USA 90*:5873 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, *Meth. Enzymol.*, *266*:460 (1996); blast.wustl/edu/blast/README.html. WU-BLAST-2 uses several search parameters, which are preferably set to the default values. The parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

An additional useful algorithm is gapped BLAST as reported by Altschul *et al.*, *Nucleic Acids Res. 25*:3389 (1997).

A percentage amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

In a similar manner, percent nucleic acid sequence identity is defined as the percentage of nucleotide residues in the candidate sequence that are identical with the nucleotides in the polynucleotide specifically disclosed herein.

The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer nucleotides than the polynucleotides specifically disclosed herein, it is understood that in one embodiment, the percentage of sequence identity will be determined based on the number of identical nucleotides in relation to the total number of nucleotides. Thus, for example, sequence identity of sequences shorter than a sequence specifically disclosed herein, will be determined using the number of nucleotides in the shorter sequence, in one embodiment. In percent identity calculations relative weight is not assigned to various manifestations of sequence variation, such as insertions, deletions, substitutions, *etc.*

In some embodiments, only identities are scored positively (+1) and all forms of sequence variation including gaps are assigned a value of "0," which obviates the need for a weighted scale or parameters as described below for sequence similarity calculations. Percent sequence identity can be calculated, for example, by dividing the number of matching identical residues by the total number of residues of the "shorter" sequence in the aligned region and multiplying by 100. The "longer" sequence is the one having the most actual residues in the aligned region.

The term "synthetic polynucleotide" refers to a polynucleotide sequence that does not exist in nature but instead is made by the hand of man, either chemically, or biologically (*i.e., in vitro* modified). In some embodiments of the invention, a synthetic polynucleotide may be a codon optimized human FKRP (e.g., **SEQ ID NO:7**) or it may be an optimized and CpG depleted human FKRP (e.g., **SEQ ID NO:1**; **SEQ ID NO:6**). Codon optimization may be as compared to wild type human FKRP (e.g., **SEQ ID NO:10**).

By "operably linked" or "operably associated" as used herein, it is meant that the indicated elements are functionally related to each other, and are also generally physically related. Thus, the term "operably linked" or "operably associated" as used herein, refers to nucleotide sequences on a single nucleic acid molecule that are functionally associated. Thus, a first nucleotide sequence that is operably linked to a second nucleotide sequence means a situation when the first nucleotide sequence is placed in a functional relationship with the second nucleotide sequence. For instance, a promoter is operably associated with a nucleotide sequence if the promoter effects the transcription or expression of said nucleotide sequence. Those skilled in the art will appreciate that the control sequences (*e.g.,* promoter) need not be

contiguous with the nucleotide sequence to which it is operably associated, as long as the control sequences function to direct the expression thereof. Thus, for example, intervening untranslated sequences that may be transcribed can be present between a promoter and a nucleotide sequence, and the promoter can still be considered "operably linked" to the nucleotide sequence.

A "promoter" is a nucleotide sequence that controls or regulates the transcription of a nucleotide sequence (*i.e.*, a coding sequence) that is operably associated with the promoter. The coding sequence may encode a polypeptide and/or a functional RNA. Typically, a "promoter" refers to a nucleotide sequence that contains a binding site for RNA polymerase II and directs the initiation of transcription. In general, promoters are found 5', or upstream, relative to the start of the coding region of the corresponding coding sequence. The promoter region may comprise other elements that act as regulators of gene expression.

The term "fragment," as applied to a polynucleotide, will be understood to mean a nucleotide sequence of reduced length relative to a reference nucleic acid or nucleotide sequence and comprising, consisting essentially of, and/or consisting of a nucleotide sequence of contiguous nucleotides identical or almost identical (*e.g.*, 90%, 92%, 95%, 98%, 99% identical) to the reference nucleic acid or nucleotide sequence. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of oligonucleotides having a length of at least about 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, or more consecutive nucleotides of a nucleic acid or nucleotide sequence of the invention (*e.g.*, the nucleotide sequence of **SEQ ID NO:1**, **SEQ ID NO:6** and/or **SEQ ID NO:7**, a nucleotide sequence having at least 90% identity to **SEQ ID NO:1** and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6** or **SEQ ID NO:7**).

The term "isolated" can refer to a nucleic acid, nucleotide sequence or polypeptide that is substantially free of cellular material, viral material, and/or culture medium (when produced by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Moreover, an "isolated fragment" is a fragment of a nucleic acid, nucleotide sequence or polypeptide that is not naturally occurring as a fragment and would not be found in the natural state. "Isolated" does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the polypeptide or nucleic acid in a form in which it can be used for the intended purpose.

The term "fragment," as applied to a polypeptide, will be understood to mean an amino acid sequence of reduced length relative to a reference polypeptide or amino acid sequence and comprising, consisting essentially of, and/or consisting of an amino acid sequence of contiguous amino acids identical or almost identical (*e.g.*, 90%, 92%, 95%, 98%, 99% identical) to the reference polypeptide or amino acid sequence. Such a polypeptide fragment according to the invention may be, where appropriate, included in a larger polypeptide of which it is a constituent. In some embodiments, such fragments can comprise, consist essentially of, and/or consist of peptides having a length of at least about 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, or more consecutive amino acids of a polypeptide or amino acid sequence according to the invention.

The nucleic acid of this invention can be present in a vector and such a vector can be present in a cell. A "vector" is any nucleic acid molecule for the cloning of and/or transfer of a nucleic acid into a cell. A vector may be a replicon to which another nucleotide sequence may be attached to allow for replication of the attached nucleotide sequence. A "replicon" can be any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, viral genome) that functions as an autonomous unit of nucleic acid replication *in vivo*, *i.e.*, capable of replication under its own control. Any suitable vector is encompassed in the embodiments of this invention, including, but not limited to, nonviral vectors (*e.g.*, plasmids, liposomes, electrically charged lipids (cytofectins), nucleic acid-protein complexes, poloxymers and biopolymers), viral vectors (*e.g.*, retrovirus, lentivirus, adeno-associated virus, poxvirus, alphavirus, baculovirus, vaccinia virus, herpes virus, Epstein-Barr virus, and adenovirus vectors) and synthetic biological nanoparticles (BNP) (*e.g.*, synthetically designed from different adeno-associated viruses, as well as other parvoviruses). The term "vector" includes both viral and nonviral (*e.g.*, plasmid) nucleic acid molecules for introducing a nucleic acid into a cell *in vitro*, *ex vivo*, and/or *in vivo*.

It will be apparent to those skilled in the art that any suitable vector can be used to deliver a heterologous nucleic acid of this invention. The choice of delivery vector can be made based on a number of factors known in the art, including age and species of the target host, *in vitro* vs. *in vivo* delivery, level and persistence of expression desired, intended purpose (*e.g.*, for therapy or polypeptide production), the target cell or organ, route of delivery, size of the isolated nucleic acid, safety concerns, and the like.

Suitable vectors also include virus vectors (*e.g.*, retrovirus, alphavirus; vaccinia virus; adenovirus, adeno-associated virus, or herpes simplex virus), lipid vectors, poly-lysine vectors,

synthetic polyamino polymer vectors that are used with nucleic acid molecules, such as plasmids, and the like.

Protocols for producing recombinant viral vectors and for using viral vectors for nucleic acid delivery can be found, *e.g.,* in *Current Protocols in Molecular Biology, Ausubel*, F. M. et al. (eds.) Greene Publishing Associates, (1989) and other standard laboratory manuals (*e.g.,* Vectors for Gene Therapy. In: *Current Protocols in Human Genetics*. John Wiley and Sons, Inc.: 1997).

A large number of vectors known in the art may be used to manipulate nucleic acids, incorporate response elements and promoters into genes, *etc.* For example, the insertion of the nucleic acid fragments corresponding to response elements and promoters into a suitable vector can be accomplished by ligating the appropriate nucleic acid fragments into a chosen vector that has complementary cohesive termini. Alternatively, the ends of the nucleic acid molecules may be enzymatically modified or any site may be produced by ligating nucleotide sequences (linkers) to the nucleic acid termini. Such vectors may be engineered to contain sequences encoding selectable markers that provide for the selection of cells that contain the vector and/or have incorporated the nucleic acid of the vector into the cellular genome. Such markers allow identification and/or selection of host cells that incorporate and express the proteins encoded by the marker. A "recombinant" vector refers to a viral or non-viral vector that comprises one or more heterologous nucleotide sequences (i.e., transgenes), e.g., one, two, three, four, five or more heterologous nucleotide sequences (e.g., a vector comprising the nucleotide sequence of **SEQ ID NO:1**, **SEQ ID NO:6** and/or **SEQ ID NO:7**).

In addition to a nucleic acid of interest (e.g., a synthetic polynucleotide of the invention), a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (delivery to specific tissues, duration of expression, etc.).

A vector comprising a synthetic polynucleotide of the invention may be used to infect and thereby delivering said synthetic polynucleotide to the infected cells. The exact method of introducing the synthetic polynucleotide into mammalian cells is, of course, not limited to the use of any particular type of vector. Any vector system now known or later identified may be used with the synthetic polynucleotides of this invention. Techniques are widely available for such procedures including the use of, for example, adenoviral vectors (Mitani et al., *Hum. Gene Ther.* 5:941-948, 1994), adeno-associated viral (AAV) vectors (Goodman et al., *Blood* 84:1492-1500, 1994), lentiviral vectors (Naldini et al., *Science* 272:263-267, 1996), and pseudotyped retroviral vectors (Agrawal et al., *Exper. Hematol.* 24:738-747, 1996). Also

included are chimeric viral particles, which are well known in the art and which can comprise viral proteins and/or nucleic acids from two or more different viruses in any combination to produce a functional viral vector. Chimeric viral particles of this invention can also comprise amino acid and/or nucleotide sequence of non-viral origin (*e.g.*, to facilitate targeting of vectors to specific cells or tissues and/or to induce a specific immune response).

Vectors may be introduced into the desired cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a nucleic acid vector transporter (see, e.g., Wu et al., *J. Biol. Chem. 267*:963 (1992); Wu et al., *J. Biol. Chem. 263*:14621 (1988); and Hartmut et al., Canadian Patent Application No. 2,012,311, filed Mar. 15, 1990). In various embodiments, other molecules can be used for facilitating delivery of a nucleic acid *in vivo*, such as a cationic oligopeptide (e.g., WO95/21931), peptides derived from nucleic acid binding proteins (e.g., WO96/25508), and/or a cationic polymer (*e.g.*, WO95/21931). It is also possible to introduce a vector *in vivo* as naked nucleic acid (see U.S. Patent Nos. 5,693,622, 5,589,466 and 5,580,859). Receptor-mediated nucleic acid delivery approaches can also be used (Curiel et al., *Hum. Gene Ther. 3*:147 (1992); Wu et al., *J. Biol. Chem. 262*:4429 (1987)).

Thus, administration or delivery of a synthetic polynucleotide of this invention can be achieved by any one of numerous, well-known approaches, for example, but not limited to, direct transfer of the nucleic acids, in a plasmid or viral vector, or via transfer in cells or in combination with carriers such as cationic liposomes. Such methods are well known in the art and readily adaptable for use in the methods described herein. Furthermore, these methods can be used to target certain diseases and tissues, organs and/or cell types and/or populations by using the targeting characteristics of the carrier, which would be well known to the skilled artisan. It would also be well understood that cell and tissue specific promoters can be employed in the nucleic acids of this invention to target specific tissues and cells and/or to treat specific diseases and disorders.

As used herein, the terms "protein" and "polypeptide" are used interchangeably and encompass both peptides and proteins, unless indicated otherwise.

A "fusion protein" is a polypeptide produced when two heterologous nucleotide sequences or fragments thereof coding for two (or more) different polypeptides not found fused together in nature are fused together in the correct translational reading frame. Illustrative fusion polypeptides include fusions of a polypeptide of the invention (or a fragment thereof) to all or a portion of glutathione-S-transferase, maltose-binding protein, or a reporter protein (*e.g.*,

Green Fluorescent Protein, β-glucuronidase, β-galactosidase, luciferase, *etc.*), hemagglutinin, c-myc, FLAG epitope, *etc.*

By the term "express" or "expression" of a polynucleotide coding sequence, it is meant that the sequence is transcribed, and optionally, translated. Typically, according to the present invention, expression of a coding sequence of the invention will result in production of the polypeptide of the invention. The entire expressed polypeptide or fragment can also function in intact cells without purification.

The term "adeno-associated virus" (AAV) in the context of the present invention includes without limitation AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV and any other AAV now known or later discovered. *See, e.g.,* BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers). A number of additional AAV serotypes and clades have been identified (*see, e.g.,* Gao *et al.*, (2004) *J. Virol.* 78:6381-6388), which are also encompassed by the term "AAV."

The genomic sequences of various AAV and autonomous parvoviruses, as well as the sequences of the inverted terminal repeats (ITRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature or in public databases such as the GenBank® database. *See*, *e.g.,* GenBank® Accession Numbers NC 002077, NC 001401, NC 001729, NC 001863, NC 001829, NC 001862, NC 000883, NC 001701, NC 001510, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226, AY028223, NC 001358, NC 001540, AF513851, AF513852, AY530579, AY631965, AY631966; the disclosures of which are incorporated herein in their entirety. *See also, e.g.,* Srivistava *et al.*, (1983) *J. Virol.* 45:555; Chiorini *et al.*, (1998) *J. Virol.* 71:6823; Chiorini *et al.*, (1999) *J. Virol.* 73:1309; Bantel-Schaal *et al.*, (1999) *J. Virol.* 73:939; Xiao *et al.*, (1999) *J. Virol.* 73:3994; Muramatsu *et al.*, (1996) *Virology* 221:208; Shade *et al.*, (1986) *J. Virol.* 58:921; Gao *et al.*, (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; U.S. Patent No. 6,156,303; the disclosures of which are incorporated herein in their entirety. An early description of the AAV1, AAV2 and AAV3 terminal repeat sequences is provided by Xiao, X., (1996), "Characterization of Adeno- associated virus (AAV) DNA replication and integration," Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, PA (incorporated herein it its entirety).

A "recombinant AAV vector genome" or "rAAV genome" is an AAV genome (*i.e.*, vDNA) that comprises at least one inverted terminal repeat (*e.g.*, one, two or three inverted terminal repeats) and one or more heterologous nucleotide sequences. rAAV vectors generally retain the 145 base terminal repeat(s) (TR(s)) in *cis* to generate virus; however, modified AAV TRs and non-AAV TRs including partially or completely synthetic sequences can also serve this purpose. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). The rAAV vector optionally comprises two TRs (*e.g.*, AAV TRs), which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s), but need not be contiguous thereto. The TRs can be the same or different from each other. The vector genome can also contain a single ITR at its 3' or 5' end.

The term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (i.e., mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The TR can be an AAV TR or a non-AAV TR. For example, a non-AAV TR sequence such as those of other parvoviruses (e.g., canine parvovirus (CPV), mouse parvovirus (MVM), human parvovirus B-19) or the SV40 hairpin that serves as the origin of SV40 replication can be used as a TR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the TR can be partially or completely synthetic, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski et al.

An "AAV terminal repeat" or "AAV TR" may be from any AAV, including but not limited to serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 or any other AAV now known or later discovered. An AAV terminal repeat need not have the native terminal repeat sequence (*e.g.*, a native AAV TR sequence may be altered by insertion, deletion, truncation and/or missense mutations), as long as the terminal repeat mediates the desired functions, e.g., replication, virus packaging, integration, and/or provirus rescue, and the like.

The terms "rAAV particle" and "rAAV virion" are used interchangeably here. A "rAAV particle" or "rAAV virion" comprises a rAAV vector genome packaged within an AAV capsid.

The AAV capsid structure is described in more detail in Bernard N. Fields et al., VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

The term "pharmacokinetic properties" has its usual and customary meaning and refers to the absorption, distribution, metabolism and excretion of FKRP.

As used herein, a "transformed" cell is a cell that has been transformed, transduced and/or transfected with a synthetic polynucleotide of this invention encoding an optimized human FKRP (e.g., **SEQ ID NO:7**) and/or optimized and CpG depleted human FKRP (e.g., **SEQ ID NO:1** and/or **SEQ ID NO:6**).

As used herein, the term "dystroglycanopathy" refers to a subset of muscular dystrophies involving a reduction in glycosylation of alpha-dystroglycan (α-DG). Such disorders include but are not limited to limb girdle muscular dystrophy 2I, congenital muscular dystrophy, Walker-Warburg syndrome, or muscle-eye-brain disease.

A "subject" of the invention includes any animal having or susceptible to a dystroglycanopathy for which prevention or treatment of said dystroglycanopathy is needed and/or desired, which can be treated, ameliorated or prevented by administration/delivery of a synthetic polynucleotide of the invention encoding FKRP to the subject. Such a subject is generally a mammalian subject (*e.g.,* a laboratory animal such as a rat, mouse, guinea pig, rabbit, primates, *etc.*), a farm or commercial animal (*e.g.,* a cow, horse, goat, donkey, sheep, *etc.*), or a domestic animal (*e.g.,* cat, dog, ferret, *etc.*). In particular embodiments, the subject is a primate subject, a non-human primate subject (*e.g.,* a chimpanzee, baboon, monkey, gorilla, *etc.*) or a human. Subjects of the invention can be a subject known or believed to be at risk of dystroglycanopathy for which prevention or treatment is needed and/or desired. Alternatively, a subject according to the invention can also include a subject not previously known or suspected to be at risk of dystroglycanopathy for which prevention or treatment is needed or desired. As a further option, the subject can be a laboratory animal and/or an animal model of disease. Suitable subjects include both males and females and subjects of any age, including embryonic (e.g., *in utero* or *in ovo*), infant, juvenile, adolescent, adult and geriatric subjects.

A "subject in need thereof" in the context of treatment or therapy is a subject known to have, or suspected of having or being at risk of having, a disease or disorder (e.g., dystroglycanopathy), and that is likely to benefit from the treatment or therapy, i.e., is in need thereof.

Synthetic Polynucleotides, Expression Cassettes and Vectors

One aspect of the present invention relates to a synthetic polynucleotide encoding a codon optimized and CpG(-) depleted human fukutin-related protein (FKRP-CpG(-)). In some embodiments, the invention provides a synthetic polynucleotide encoding a human fukutin-related protein (FKRP), wherein the synthetic polynucleotide comprises, consists essentially of, or consists of: the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence

having at least 90% sequence identity (e.g., about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to **SEQ ID NO:1**.

The present inventors have surprisingly discovered that delivery of a vector comprising a synthetic polynucleotide encoding CpG(-) depleted human fukutin-related protein (FKRP-CpG(-)) provided a safer immune profile when compared to a codon optimized human FKRP (**SEQ ID NO:8**) in the same vector.

In some embodiments, a synthetic polynucleotide encoding FKRP (e.g., polynucleotide encoding **SEQ ID NO:1** and/or a polynucleotide having at least about 90% identity to **SEQ ID NO:1** may be operably associated with control or regulatory sequences. For example, a synthetic polynucleotide of this invention may be operably associated with expression control elements, such as promoters, transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites (IRES), enhancers, and the like. In some embodiments, the synthetic polynucleotide encoding FKRP (e.g., polynucleotide encoding **SEQ ID NO:1** and/or a polynucleotide having at least about 90% identity to **SEQ ID NO:1** may be operably associated with for example a poly A tail (see, e.g., **SEQ ID NO:6**).

In some embodiments, a synthetic polynucleotide encoding a codon optimized and CpG(-) depleted human fukutin-related protein (FKRP-CpG(-)) comprises, consists essentially of, or consists of: the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% sequence identity (e.g., about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical) to **SEQ ID NO:6** (e.g., an optimized and CpG depleted FKRP (**SEQ ID NO:1**) operably linked to a poly A tail having the nucleotide sequence of **SEQ ID NO:5**)

In some embodiments, the present invention provides a synthetic polynucleotide encoding a human fukutin-related protein (FKRP)that comprises, consists essentially of, or consists of: the nucleotide sequence of **SEQ ID NO:7** and/or a nucleotide sequence having at least 80% identity to the nucleotide sequence of **SEQ ID NO:7**.

Those skilled in the art will appreciate that a variety of promoter/enhancer elements may be used depending on the level and tissue-specific expression desired. The promoter/enhancer may be constitutive or inducible, depending on the pattern of expression desired. The promoter/enhancer may be native/endogenous or foreign/heterologous and can be a natural or a synthetic sequence. By foreign/heterologous, it is intended that the transcriptional initiation region is not found in the wild-type host into which the transcriptional initiation region is introduced.

Promoter/enhancer elements can be native/endogenous to the target cell or subject to

be treated and/or native to the heterologous nucleic acid sequence. The promoter/enhancer element is generally chosen so that it will function in the target cell(s) of interest. In representative embodiments, the promoter/enhancer element is a mammalian promoter/enhancer element. The promoter/enhance element may be constitutive or inducible.

Inducible expression control elements are generally used in those applications in which it is desirable to provide regulation over expression of the heterologous nucleic acid sequence(s). Inducible promoter/enhancer elements include, but are not limited to, hormone-inducible and metal-inducible elements.

Promoters/enhancer elements for gene delivery can be tissue-specific or tissue-preferred promoter/enhancer elements, and include muscle specific or preferred (including cardiac, skeletal and/or smooth muscle), neural tissue specific or preferred (including brain-specific), eye (including retina-specific and cornea-specific), bone marrow specific or preferred, pancreatic specific or preferred, spleen specific or preferred, and lung specific or preferred promoter/enhancer elements.

Thus, any promoter operable in the organism or subject in which expression of a synthetic polynucleotide of the invention encoding a FKRP is desired can be used. Promoters useful with this invention include, but are not limited to, a creatine kinase (CK) promoter, a chicken β-actin promoter (CB), desmine promoter, α actin promoter, or any viral promoter. In representative embodiments, the promoter can be a CK7 promoter. In some embodiments, a promoter can be modified to include other regulatory elements, for example, enhancer sequences. In other embodiments, the promoters are not modified to include other regulatory elements such as enhancers.

In some embodiments, a promoter operably associated with a synthetic polynucleotide of this invention may be a muscle specific promoter. Muscle specific promoters useful with this invention can include, but are not limited to, a creatine kinase (CK) promoter and/or a synthetic muscle specific promoter. In some embodiments, a synthetic muscle specific promoter may be a Syn 100 promoter, optionally wherein the Syn100 promoter may comprise a nucleotide sequences having at least 90% identity to **SEQ ID NO:2.**

In some embodiments, an enhancer sequence useful with this invention can include a CMV enhancer, SV40 enhancer, a muscle creatine kinase enhancer, and/or a myosin light chain enhancer, troponin promoter, tropomycin enhancer, and/or any other synthetic enhancer with or without modification. In some embodiments, an enhancer sequence operably associated with a synthetic polynucleotide of this invention may be an intron, optionally a Vh4-Ig-intron3. In

some embodiments, a Vh4-Ig-intron3 useful with this invention can comprise the nucleotide sequence of **SEQ ID NO:3**.

In some embodiments, an expression system or construct can include a 3' untranslated region downstream of the nucleotide sequence encoding the desired recombinant protein. This region can increase expression of the transgene. Among the 3' untranslated regions useful in this regard are sequences that provide a poly A signal. In some embodiments, a poly A tail may be from bovine growth hormone. In some embodiments, the poly A tail from bovine growth hormone may comprise the nucleotide sequence of **SEQ ID NO:4**. In some embodiments, the poly A tail may comprise the nucleotide sequence of **SEQ ID NO:5**.

In some embodiments, a synthetic polynucleotide of the present invention may be comprised in a vector or expression cassette wherein the synthetic polynucleotide is operably linked to expression control elements as described herein (see, as an example, **SEQ ID NO:7**).

As used herein, the term "vector," "virus vector," "delivery vector" (and similar terms) generally refers to a virus particle that functions as a nucleic acid delivery vehicle, and which comprises the viral nucleic acid (*i.e.*, the vector genome) packaged within the virion. Virus vectors according to the present invention comprise a chimeric AAV capsid according to the invention and can package an AAV or rAAV genome or any other nucleic acid including viral nucleic acids. Alternatively, in some contexts, the term "vector," "virus vector," "delivery vector" (and similar terms) may be used to refer to the vector genome (*e.g.*, vDNA) in the absence of the virion and/or to a viral capsid that acts as a transporter to deliver molecules tethered to the capsid or packaged within the capsid.

The virus vectors of the invention can further be duplexed parvovirus particles (double stranded or self-complementary) as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety), wherein double stranded (duplex) genomes can be packaged. Thus, in some embodiments, a viral vector (e.g., AAV) may be single stranded. In some embodiments, a viral vector (e.g., AAV) may be double stranded or self-complementary.

A "recombinant AAV vector genome" or "rAAV genome" is an AAV genome (*i.e.*, vDNA) that comprises at least one inverted terminal repeat (*e.g.*, one, two or three inverted terminal repeats) and one or more heterologous nucleotide sequences. rAAV vectors generally retain the 145 base terminal repeat(s) (TR(s)) in *cis* to generate virus; however, modified AAV TRs and non-AAV TRs including partially or completely synthetic sequences can also serve this purpose. All other viral sequences are dispensable and may be supplied in *trans* (Muzyczka, (1992) *Curr. Topics Microbiol. Immunol.* 158:97). The rAAV vector optionally

comprises two TRs (*e.g.*, AAV TRs), which generally will be at the 5' and 3' ends of the heterologous nucleotide sequence(s), but need not be contiguous thereto. The TRs can be the same or different from each other. The vector genome can also contain a single ITR at its 3' or 5' end.

The term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that forms a hairpin structure and functions as an inverted terminal repeat (*i.e.*, mediates the desired functions such as replication, virus packaging, integration and/or provirus rescue, and the like). The TR can be an AAV TR or a non-AAV TR. For example, a non-AAV TR sequence such as those of other parvoviruses (*e.g.*, canine parvovirus (CPV), mouse parvovirus (MVM), human parvovirus B-19) or the SV40 hairpin that serves as the origin of SV40 replication can be used as a TR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Further, the TR can be partially or completely synthetic, such as the "double-D sequence" as described in United States Patent No. 5,478,745 to Samulski *et al.* In some embodiments of the invention, a viral vector useful with this invention may comprise a mutated terminal repeat (ITR).

Parvovirus genomes have palindromic sequences at both their 5' and 3' ends. The palindromic nature of the sequences leads to the formation of a hairpin structure that is stabilized by the formation of hydrogen bonds between the complementary base pairs. This hairpin structure is believed to adopt a "Y" or a "T" shape. See, e.g., Fields et al., VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

In some embodiments, a viral vector useful with this invention is an AAV vector from any known AAV serotype including without limitation AAV type 1, AAV type 2, AAV type 3 (including types 3A and 3B), AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV and any other AAV now known or later discovered. In some embodiments, the AAV vector is an AAV8 vector. In some embodiments, the AAV vector is an AAV9 vector.

In some embodiments, the AAV vector may be modified. Thus, for example, an AAV capsid protein of a virus vector can comprise a modification in the amino acid sequence in the three-fold axis loop 4 (Opie et al., *J. Virol.* 77: 6995-7006 (2003)). Such modifications have been shown to confer one or more desirable properties to virus vectors comprising the modified AAV capsid protein including without limitation (i) reduced transduction of liver, (ii) enhanced movement across endothelial cells, (iii) systemic transduction; (iv) enhanced transduction of muscle tissue (*e.g.*, skeletal muscle, cardiac muscle and/or diaphragm muscle), and/or (v)

reduced transduction of brain tissues (e.g., neurons). Thus, in representative embodiments, such modifications can reduce delivery of the synthetic polynucleotides of the invention to the liver (See, Asokan et al. *Nat Biotechnol.* 28(1):79–82 (2010); U.S. Patent No. 8,889,641).

In some embodiments, the vector may further comprise a nucleic acid element that reduces expression in the liver. In some embodiments, a vector may further comprise a mir122 binding element. The mir122 sequence and its use to reduce expression in the liver is well known in the art (See, e.g., Qiao et al, *Gene Therapy* 18, 403-410 (April 2011) doi:10.1038/gt.2010.157).

A vector can further encode reporter polypeptides (e.g., an enzyme). Reporter polypeptides are known in the art and include, but are not limited to, a fluorescent protein (e.g., EGFP, GFP, RFP, BFP, YFP, or dsRED2), an enzyme that produces a detectable product, such as luciferase (e.g., from *Gaussia*, *Renilla*, or *Photinus*), β-galactosidase, β-glucuronidase, alkaline phosphatase, and chloramphenicol acetyltransferase gene, or proteins that can be directly detected. Virtually any protein can be directly detected by using, for example, specific antibodies to the protein. Additional markers (and associated antibiotics) that are suitable for either positive or negative selection of eukaryotic cells are disclosed in Sambrook and Russell (2001), *Molecular Cloning*, 3rd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., and Ausubel *et al.* (1992), *Current Protocols in Molecular Biology*, John Wiley & Sons, including periodic updates.

A further aspect of the invention relates to a cell comprising at least one synthetic polynucleotide of the invention and/or vector comprising the at least one synthetic polynucleotide of the invention (*e.g.,* an isolated cell, a transformed cell, a recombinant cell, etc.). Thus, various embodiments of the invention are directed to recombinant host cells comprising a vector (*e.g.,* expression cassette) including the synthetic polynucleotide of the invention. Such a cell may be isolated and/or present in an animal, *e.g.,* a transgenic animal. Transformation of cells is described further below.

Recombinant virus vectors according to the present invention find use in both veterinary and medical applications. Suitable subjects include both avians and mammals. The term "avian" as used herein includes, but is not limited to, chickens, ducks, geese, quail, turkeys, pheasant, parrots, parakeets. The term "mammal" as used herein includes, but is not limited to, humans, primates, non-human primates (e.g., monkeys and baboons), rodents (e.g., rats, rabbits, mice, hamsters, guinea pigs and the like), cattle, sheep, goats, pigs, horses, cats, dogs, etc. Human subjects include neonates, infants, juveniles, and adults. Optionally, the

subject is "in need of" the methods of the present invention, e.g., because the subject has or is believed at risk for a disorder including those described herein or that would benefit from the delivery of a polynucleotide including those described herein. In some embodiments, the subject may be a laboratory animal and/or an animal model of disease.

Thus, in some embodiments, the invention provides a transgenic animal comprising a synthetic polynucleotide of the invention, and/or an expression cassette, vector, and/or transformed cell comprising the same. In some embodiments, the transgenic animal is not a human.

A transgenic animal may be produced by introducing into a single cell embryo the synthetic polynucleotide of the invention encoding FKRP (e.g., a nucleotide sequence encoding FKRP that is codon optimized and CpG depleted, the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6**, the nucleotide sequence of **SEQ ID NO:7**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:7** in a manner such that the synthetic polynucleotide is stably integrated into the DNA of germ line cells of the mature animal, and is inherited in normal Mendelian fashion. The transgenic animal of this invention would have a phenotype of producing FKRP in body fluids and/or tissues. In some embodiments, the FKRP may be removed from these fluids and/or tissues and processed, for example for therapeutic use. (See, e.g., *Clark et al.* "Expression of human anti-hemophilic factor IX in the milk of transgenic sheep" *Bio/Technology* 7:487-492 (1989); Van Cott et al. "Haemophilic factors produced by transgenic livestock: abundance can enable alternative therapies worldwide" *Haemophilia* 10(4):70-77 (2004), the entire contents of which are incorporated by reference herein).

DNA molecules can be introduced into embryos by a variety of means including but not limited to microinjection, calcium phosphate mediated precipitation, liposome fusion, or retroviral infection of totipotent or pluripotent stem cells. The transformed cells can then be introduced into embryos and incorporated therein to form transgenic animals. Methods of making transgenic animals are described, for example, in Transgenic Animal Generation and Use by L. M. Houdebine, Harwood Academic Press, 1997. Transgenic animals also can be generated using methods of nuclear transfer or cloning using embryonic or adult cell lines as described for example in Campbell et al., *Nature* 380:64-66 (1996) and Wilmut et al., *Nature* 385:810-813 (1997). Further a technique utilizing cytoplasmic injection of DNA can be used as described in U.S. Pat. No. 5,523,222.

FKRP-producing transgenic animals can be obtained by introducing a chimeric construct comprising a synthetic polynucleotide of the invention (e.g., a nucleotide sequence encoding FKRP that is codon optimized and CpG depleted, the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6**, the nucleotide sequence of **SEQ ID NO:7**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:7**). Methods for obtaining transgenic animals are well-known. See, for example, Hogan *et al.*, Manipulating the Mouse Embryo, (Cold Spring Harbor Press 1986); Krimpenfort *et al.*, *Bio/Technology* 9:88 (1991); Palmiter *et al.*, *Cell* 41:343 (1985), Kraemer *et al.*, Genetic Manipulation of the Early Mammalian Embryo, (Cold Spring Harbor Laboratory Press 1985); Hammer *et al.*, *Nature* 315:680 (1985); Wagner *et al.*, U.S. Pat. No. 5,175,385; Krimpenfort *et al.*, U.S. Pat. No. 5,175,384, Janne *et al.*, *Ann. Med.* 24:273 (1992), Brem *et al.*, *Chim. Oggi.* 11:21 (1993), Clark *et al.*, U.S. Pat. No. 5,476,995, all incorporated by reference herein in their entireties.

A synthetic polynucleotide of this invention encoding FKRP, or vector and/or cell comprising the synthetic polynucleotide can be included in a pharmaceutical composition. Some embodiments are directed to a kit which includes said synthetic polynucleotide, or vector and/or cell comprising said synthetic polynucleotide of the invention and/or reagents and/or instructions for using the kit, *e.g.,* to carry out the methods of this invention.

In some embodiments, the present invention provides a pharmaceutical composition comprising a synthetic polynucleotide of the invention and/or expression cassette, vector, and/or transformed cell comprising the same in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and will preferably be in solid or liquid particulate form.

By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable biological effects.

A further aspect of the invention relates to the use of the synthetic polynucleotides encoding FKRP, or vector, expression cassette, and/or cell comprising one or more synthetic polynucleotides encoding FKRP. Thus, one aspect relates to a method of producing a FKRP polypeptide in a cell or in a subject, comprising delivering to the cell or the subject a synthetic polynucleotide of the invention and/or a vector, and/or transformed cell comprising the same,

thereby producing the FKRP polypeptide in said cell or said subject. The synthetic polynucleotide, vector, and/or transformed cell are delivered under conditions whereby expression of the synthetic polynucleotide encoding FKRP occurs to produce a FKRP polypeptide. Such conditions are well known in the art.

Methods

One aspect of the present invention is a method of transferring a synthetic polynucleotide of this invention to a cell *in vitro*. The synthetic polynucleotide and/or expression cassette and/or vector comprising the same may be introduced to the cells in the appropriate amount. The virus vector may be introduced to the cells at the appropriate multiplicity of infection according to standard transduction methods appropriate for the particular target cells. Titers of the virus vector or capsid to administer can vary, depending upon the target cell type and number, and the particular virus vector or capsid, and can be determined by those of skill in the art without undue experimentation. In particular embodiments, at least about 10^3 infectious units, more preferably at least about 10^3, 10^4, 10^5 or 10^6 infectious units are introduced to the cell.

In some embodiments, the invention provides a method of delivering/administering a nucleic acid to a cell comprising, consisting essentially of, or consisting of: delivering to the cell a synthetic polynucleotide of the invention (e.g., a nucleotide sequence encoding FKRP that is codon optimized and CpG depleted, the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6**, the nucleotide sequence of **SEQ ID NO:7**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:7**); and/or a vector comprising, consisting essentially of, or consisting of the same. In some embodiments, the synthetic polynucleotide of the invention, and/or vector may be administered to the cell in a therapeutically effective amount.

Another aspect of the invention provides a method of delivering/administering a nucleic acid to a subject in need thereof, the method comprising, consisting essentially of, or consisting of: delivering to the subject a synthetic polynucleotide of the invention (e.g., a nucleotide sequence encoding FKRP that is codon optimized and CpG depleted, the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6**, the nucleotide sequence of **SEQ ID NO:7**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:7**); and/or a vector and/or a transformed cell comprising, consisting essentially of, or consisting of the same. In some embodiments, the

synthetic polynucleotide of the invention, and/or vector and/or transformed cell comprising the same may be delivered to the subject in a therapeutically effective amount.

Administration of the virus vectors of the present invention to a human subject or an animal in need thereof can be by any means known in the art. In some embodiments, the virus vector is delivered in an effective dose in a pharmaceutically acceptable carrier.

The present invention further provides a method of treating a dystroglycanopathy in a subject in need thereof, the method comprising, consisting essentially of, or consisting of: delivering/administering to said subject a synthetic polynucleotide of the invention (e.g., a nucleotide sequence encoding FKRP that is codon optimized and CpG depleted, the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6**, the nucleotide sequence of **SEQ ID NO:7**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:7**); and/or a vector and/or a transformed cell comprising the same, wherein the synthetic polynucleotide is expressed in the subject, thereby treating dystroglycanopathy in the subject.

In some embodiments, the dystroglycanopathy comprises, consists essentially of, or consists of a mutation in the native or endogenous nucleic acid encoding FKRP and/or a deficiency in glycosylation of alpha-dystroglycan (α-DG), or any combination thereof. In some embodiments, the dystroglycanopathy can include, but is not limited to, limb girdle muscular dystrophy 2I, congenital muscular dystrophy, Walker-Warburg syndrome, muscle-eye-brain disease, and/or any combination thereof.

The present invention further provides a method of reducing serum creatine kinase levels in a subject in need thereof, the method comprising delivering to the subject a synthetic polynucleotide of the invention (e.g., a double stranded vector comprising, for example, an optimized human FKRP or an optimized human FKRP that is CpG depleted as described herein; e.g., **SEQ ID NO:1**, **SEQ ID NO:6**, **SEQ ID NO:7**) and/or a vector and/or a transformed cell comprising, consisting essentially of, or consisting of the same, wherein the synthetic polynucleotide is expressed in the subject, thereby producing human FKRP and reducing serum creatine kinase levels in the subject.

In further aspects, the present invention providers nucleic acid constructs encoding FKRP that provide a safer immune response when delivered to a subject. Thus, in some embodiments, a method of reducing an immune response when administering an FKRP expressing vector is provided, the method comprising administering to the subject a synthetic polynucleotide of the invention (e.g., an optimized human FKRP that is CpG depleted; e.g.,

SEQ ID NO:1, **SEQ ID NO:6**) and/or a vector and/or a transformed cell comprising the same, wherein the synthetic polynucleotide is expressed in the subject, thereby reducing the immune response when compared to administering a codon optimized human FKRP expressing vector (e.g., as compared to **SEQ ID NO:8**). In some embodiments, reducing an immune response may comprise reduced IL17A levels as compared to a subject having been administered a vector expressing an optimized human FKRP having the nucleotide sequence of **SEQ ID NO:8** (UNC-0090 opt huFKRP).

In embodiments of the invention, the dosage of a vector (*e.g.*, a viral vector or other nucleic acid vector) encoding a synthetic FKRP of this invention (*e.g.*, comprising, consisting essentially of, or consisting of a nucleotide sequence encoding FKRP that is codon optimized and CpG depleted, the nucleotide sequence of **SEQ ID NO:1**; and/or a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**, the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:6**, the nucleotide sequence of **SEQ ID NO:7**; and/or a nucleotide sequence having at least 80% identity to **SEQ ID NO:7**) can be in an amount such that a therapeutic plasma concentration of FKRP is achieved. In some embodiments, the dosage may be about 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^3, 10^{14}, 10^{15}, 10^{16} transducing units or more, e.g., about 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} transducing units, e.g., about 10^{11}, 10^{12}, 10^{13}, 10^{14} or 10^{15} transducing units. In some embodiments, the dosage may be about $1x10^{10}$ vector particles/per kg body weight to about $1x10^{15}$ vector particles/per kg body weight (*e.g.*, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} vector particles/per kg body weight, and any range or value therein). In some embodiments, the dosage can be about $1x10^{12}$ vector particles/per kg body weight to about $1x10^{15}$ vector particles/per kg body weight (e.g., 10^{12}, 10^{13}, 10^{14}, 10^{15} vector particles/per kg body weight, and any range or value therein). Doses and virus titer transducing units may be calculated as vector or viral genomes (vg). One of skill in the art would be able to determine the optimal dose for a given subject and a given condition.

In some embodiments, the present invention provides a pharmaceutical composition comprising a virus vector of the invention in a pharmaceutically acceptable carrier and, optionally, other medicinal agents, pharmaceutical agents, stabilizing agents, buffers, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid. For other methods of administration, the carrier may be either solid or liquid. For inhalation administration, the carrier will be respirable, and will preferably be in solid or liquid particulate form.

By "pharmaceutically acceptable" it is meant a material that is not toxic or otherwise undesirable, *i.e.*, the material may be administered to a subject without causing any undesirable

biological effects. A "pharmaceutically acceptable" component such as a salt, carrier, excipient or diluent of a composition according to the present invention is a component that (i) is compatible with the other ingredients of the composition in that it can be combined with the compositions of the present invention without rendering the composition unsuitable for its intended purpose, and (ii) is suitable for use with subjects as provided herein without undue adverse side effects (such as toxicity, irritation, and allergic response). Side effects are "undue" when their risk outweighs the benefit provided by the composition. Non-limiting examples of pharmaceutically acceptable components include, without limitation, any of the standard pharmaceutical carriers such as phosphate buffered saline solutions, water, emulsions such as oil/water emulsion, microemulsions and various types of wetting agents. In certain embodiments, the pharmaceutically acceptable carrier is sterile and would be deemed suitable for administration into human subjects according to regulatory guidelines for pharmaceutical compositions comprising the carrier.

One aspect of the present invention is a method of transferring a synthetic polynucleotide of the invention to a cell *in vitro*. The virus vector may be introduced to the cells at the appropriate multiplicity of infection according to standard transduction methods appropriate for the particular target cells. Titers of the virus vector to administer can vary, depending upon the target cell type and number, and the particular virus vector can be determined by those of skill in the art without undue experimentation. In particular embodiments, at least about 1 infectious unit, more preferably at least about 2 or more infectious units are introduced to one target cell.

Cell(s) into which the polynucleotide, expression cassette, and/or vector of the invention, *e.g.*, virus vector, can be introduced may be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells such as neurons, oligodendrocytes, glial cells, astrocytes), lung cells, cells of the eye (including retinal cells, retinal pigment epithelium, and corneal cells), epithelial cells (*e.g.*, gut and respiratory epithelial cells), skeletal muscle cells (including myoblasts, myotubes and myofibers), diaphragm muscle cells, dendritic cells, pancreatic cells (including islet cells), hepatic cells, a cell of the gastrointestinal tract (including smooth muscle cells, epithelial cells), heart cells (including cardiomyocytes), bone cells (*e.g.*, bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, joint cells (including, *e.g.*, cartilage, meniscus, synovium and bone marrow), germ cells, and the like. Alternatively, the cell may be any progenitor cell. As a further alternative, the cell can be a stem cell (*e.g.*, neural stem cell). As still a further alternative, the cell may be a

cancer or tumor cell (cancers and tumors are described above). Moreover, the cells can be from any species of origin, as indicated above.

A synthetic polynucleotide of the invention, and/or expression cassettes, and/or vectors, e.g., virus vector, comprising the same may be introduced to cells *in vitro* for the purpose of administering the modified cell to a subject. In particular embodiments, the cells have been removed from a subject, and the synthetic polynucleotide, and/or expression cassette and/or vector of the invention (e.g., virus vector) comprising the synthetic polynucleotide are introduced therein, and the cells are then replaced back into the subject. Methods of removing cells from subject for treatment *ex vivo*, followed by introduction back into the subject are known in the art (*see, e.g.*, U.S. patent No. 5,399,346). Alternatively, the synthetic polynucleotide of the invention, and/or expression cassette, and/or vector (e.g., virus vector) may be introduced into cells from another subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof.

Suitable cells for *ex vivo* gene therapy are as described above. Dosages of the cells to administer to a subject will vary upon the age, condition and species of the subject, the type of cell, the nucleic acid being expressed by the cell, the mode of administration, and the like. Typically, at least about 10^2 to about 10^8 or about 10^3 to about 10^6 cells will be administered per dose in a pharmaceutically acceptable carrier. In particular embodiments, the cells transduced with the virus vector are administered to the subject in an effective amount in combination with a pharmaceutical carrier.

An effective amount of a composition of this invention will vary from composition to composition and subject to subject, and will depend upon a variety of factors such as age, species, gender, weight, overall condition of the subject, and the particular disease or disorder to be treated. An effective amount can be determined in accordance with routine pharmacological procedures know to those of ordinary skill in the art. In some embodiments, a dose ranging from about $1x10^{12}$ vector particles/per kg body weight to about $1x10^{15}$ vector particles/per kg body weight will have therapeutic efficacy. In embodiments employing viral vectors for delivery of the nucleic acid of this invention, viral doses can be measured to include a particular number of virus particles or plaque forming units (pfu) or infectious particles, depending on the virus employed. For example, in some embodiments, particular unit doses can include about 10^3 to about 10^{16} pfu or infectious particles per kg body weight (e.g., about 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15} or 10^{16} pfu or infectious particles per kg body weight), or any range or value therein. Further exemplary doses for achieving therapeutic effects are virus titers can be at least about 10^5 to about 10^{15} transducing

units per kg body weight (e.g., at least about 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^3, 10^{14}, 10^{15} transducing units per kg body weight) or any range or value therein. In representative embodiments, doses for achieving therapeutic effects are virus titers can be at least about 10^8 to about 10^{15} transducing units per kg body weight (e.g., about 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13} or 10^{14}, 10^{15} transducing units per kg body weight) or any range or value therein. Doses and virus titer transducing units may be calculated as vector or viral genomes (vg). As the skilled artisan would understand, the specific dose would depend on the size of the target/subject and the nature of the target/subject.

The frequency of administration of a composition of this invention can be as frequent as necessary to impart the desired therapeutic effect. For example, the composition can be administered one, two, or more times per day, one, two, three, four or more times a week, one, two, three, four or more times a month, one, two, three or four times a year and/or as necessary to control a particular condition and/or to achieve a particular effect and/or benefit. In particular embodiments, more than one administration (e.g., two, three, four or more administrations) may be employed to achieve the desired level of gene expression over a period of various intervals, e.g., daily, weekly, monthly, yearly, etc. In some embodiments, one, two, three or four doses over the lifetime of a subject can be adequate to achieve the desired therapeutic effect. The amount and frequency of administration of the composition of this invention will vary depending on the particular condition being treated or to be prevented and the desired therapeutic effect.

Exemplary modes of administration include oral, rectal, transmucosal, topical, intranasal, inhalation (e.g., via an aerosol), buccal (e.g., sublingual), vaginal, intrathecal, intraocular, *in utero* (or *in ovo*), parenteral (*e.g.,* intravenous, subcutaneous, intradermal, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, and intraarticular), topical (e.g., to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), and the like, as well as direct tissue or organ injection (e.g., to skeletal muscle, cardiac muscle, diaphragm muscle or brain). The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular vector that is being used. In representative embodiments, the route of delivery for treatment of dystroglycanopathy is intramuscular, intravenous and intraarterial to a part of or the whole body.

Delivery to any of these tissues can also be achieved by delivering a depot comprising the virus vector, which can be implanted into the tissue or the tissue can be contacted with a film or other matrix comprising the virus vector. Examples of such implantable matrices or

substrates are described in U.S. Patent No. 7,201,898.

Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus vector in a local rather than systemic manner, for example, in a depot or sustained-release formulation. Further, the virus vector can be delivered dried to a surgically implantable matrix such as a bone graft substitute, a suture, a stent, and the like (*e.g.*, as described in U.S. Patent 7,201,898).

Pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of the composition of this invention; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Oral delivery can be performed by complexing a virus vector of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers include plastic capsules or tablets, as known in the art. Such formulations are prepared by any suitable method of pharmacy, which includes the step of bringing into association the composition and a suitable carrier (which may contain one or more accessory ingredients as noted above). In general, the pharmaceutical composition according to embodiments of the present invention are prepared by uniformly and intimately admixing the composition with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture. For example, a tablet can be prepared by compressing or molding a powder or granules containing the composition, optionally with one or more accessory ingredients. Compressed tablets are prepared by compressing, in a suitable machine, the composition in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, and/or surface active/dispersing agent(s). Molded tablets are made by molding, in a suitable machine, the powdered compound moistened with an inert liquid binder.

Pharmaceutical compositions suitable for buccal (sub-lingual) administration include lozenges comprising the composition of this invention in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia.

Pharmaceutical compositions suitable for parenteral administration can comprise sterile aqueous and non-aqueous injection solutions of the composition of this invention, which preparations are optionally isotonic with the blood of the intended recipient. These preparations can contain anti-oxidants, buffers, bacteriostats and solutes, which render the

composition isotonic with the blood of the intended recipient. Aqueous and non-aqueous sterile suspensions, solutions and emulsions can include suspending agents and thickening agents. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The compositions can be presented in unit/dose or multi-dose containers, for example, in sealed ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water-for-injection immediately prior to use.

Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described. For example, an injectable, stable, sterile composition of this invention in a unit dosage form in a sealed container can be provided. The composition can be provided in the form of a lyophilizate, which can be reconstituted with a suitable pharmaceutically acceptable carrier to form a liquid composition suitable for injection into a subject. The unit dosage form can be from about 1 μg to about 10 grams of the composition of this invention. When the composition is substantially water-insoluble, a sufficient amount of emulsifying agent, which is physiologically acceptable, can be included in sufficient quantity to emulsify the composition in an aqueous carrier. One such useful emulsifying agent is phosphatidyl choline.

Pharmaceutical compositions suitable for rectal administration can be presented as unit dose suppositories. These can be prepared by admixing the composition with one or more conventional solid carriers, such as for example, cocoa butter and then shaping the resulting mixture.

Pharmaceutical compositions of this invention suitable for topical application to the skin can take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers that can be used include, but are not limited to, petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof. In some embodiments, for example, topical delivery can be performed by mixing a pharmaceutical composition of the present invention with a lipophilic reagent (*e.g.,* DMSO) that is capable of

passing into the skin.

Pharmaceutical compositions suitable for transdermal administration can be in the form of discrete patches adapted to remain in intimate contact with the epidermis of the subject for a prolonged period of time. Compositions suitable for transdermal administration can also be delivered by iontophoresis *(see,* for example, *Pharm. Res.* 3:318 (1986)) and typically take the form of an optionally buffered aqueous solution of the composition of this invention. Suitable formulations can comprise citrate or bis\tris buffer (pH 6) or ethanol/water and can contain from 0.1 to 0.2M active ingredient.

The virus vectors disclosed herein may be administered to the lungs of a subject by any suitable means, for example, by administering an aerosol suspension of respirable particles comprised of the virus vectors, which the subject inhales. The respirable particles may be liquid or solid. Aerosols of liquid particles comprising the virus vectors may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. See, e.g., U.S. Patent No. 4,501,729. Aerosols of solid particles comprising the virus vectors may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

Having described the present invention, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the invention.

EXAMPLES

Example 1. Double-stranded AAV vector containing optimized hu-FKRP gene codon.

The AAV vector is based on a single-stranded (ss) DNA virus. Its transgene expression requires the conversion of ssDNA to double-stranded (ds) genome, a rate limiting step for AAV transduction. The limitation of using ds-AAV vector is that it only contains half size of the single-stranded AAV genome.

Specifically, in this study, AAV9 vectors containing opti-hu-FKRP gene driven by different promoters were delivered into 2 to 3 months old *E310^{stop}/L276I* mice via tail vein injection at the dosage of 4 x 10^13 vg/kg. The four groups were: CK group which was ss-AAV containing muscle-specific creatine-kinase based promoter*(Vannoy et al. Mol. Ther. Methods Clin Dev.* 11:106-120 (2018); Qiao et al. 2014. *Mol Ther* 22:1890-9); CK-Mir122T group which was ss-AAV containing CK promoter and liver de-targeting liver-specific micro-RNA Mir122T sequences(Qiao et al, *Gene Therapy* 18, 403-410 (April 2011)); CB group which was ss-AAV containing ubiquitous CB promoter (CMV enhancer/chicken β-actin

promoter) (Qiao et al. 2014. *Mol Ther* 22:1890-9); ds-syn100 group which was double-stranded AAV vector containing a synthetic muscle promoter and an 100 bp fragment downstream of the +1 site in the chicken skeletal α-actin promoter (gcggcggc ccacgagctacccggaggagcgggaggcgtctctgccagcggtccgacgcgcagtcagcaccaggtaggtgggcaccgcgccgt gccgtgcc (**SEQ ID NO:9**))(Qiao et al. 2014. *Mol Ther* 22:1890-9).

Before injection, the serum CK was measured to make sure onset of the dystrophic phenotype. We measured serum CK periodically post treatment at 3 months, 8 months, and 15 months post injection. The average CK level of all mice before treatment was 4688±3718 U/L. At three months post injection, the AAV9-CK-Mir122T group (493 ± 492 U/L, n= 7, $p<0.05$), the AAV9-CB group (134 ± 78 U/L, n = 7, $p<0.01$), and the AAV9-ds-syn100 group (214 ± 122 U/L) all demonstrated significant decrease of serum CK level as compared to that of the untreated group (1498 ± 1204, n = 11) (**FIG. 1**). The AAV9-CK group (612 ± 337 U/L, $p>0.05$, n = 6) demonstrated the trend of decrease in serum CK level without statistical significance as compared to the untreated control group.

At 8 months post injection, the AAV9-CB group (245 ± 313, n=4, $p<0.05$) and the AAV9-ds-syn100 group (412 ± 525, n = 5, $p<0.05$) demonstrated significant decrease; while the AAV9-CK-Mir122T group (616 ± 912 U/L, n = 4, $p>0.05$) and the AAV9-CK group (666 ± 572, n=5, $p>0.05$) only displayed the trend of decrease in serum CK level as compared to the untreated group (1576 ± 869 U/L, n=6). At 15 months post injection, all treated group demonstrated the trend of decrease in serum CK level without statistical significance because of the limited n number. Specifically, the serum CK level of the AAV9-CB group was 332±466 U/L (n = 3), the AAV9-ds-syn100 group was 280±415 U/L (n=4), the AAV9-CK-Mir122T group was 454 ± 373 U/L (n=3), the AAV9-CK group was 954±1055 U/L (n=4), and the control untreated group was 1581±1856 U/L (n=7) (**FIG. 1**).

Additionally, we performed muscle function test by treadmill and grip force measurement at 12 months post treatment. For the treadmill test, the AAV9-ds-syn100 group (464±132 meters, n=4, $p<0.05$), the AAV9-CK-Mir122T group (496±142 meters, n=4, $p<0.05$), and the AAV9-CB group (482±42 meters, n=3, $p<0.01$) ran significant longer distance than the untreated control group (303±53meters, n=6). The AAV9-CK group (419±132meters, n=5, $p = 0.07$) indeed ran longer distance but lack statistical significance as compared to the untreated control group. For the grip force measurement, only the AAV9-ds-syn100 group (4.28±0.56 g/body weight g, n=5, $p<0.05$) demonstrated significant stronger strength than the untreated control group (3.6±0.7 g/body weight g, n=7) (**FIG. 2**). Together, these experiments showed that a double-stranded AAV vector containing optimized hu-FKRP gene driven by

muscle-specific synthetic promoter syn100 provided superior therapeutic effects showing reduced serum creatine kinase level, increased treadmill running distance, and improved grip strength when compared to the single-stranded AAV vector comprising the same codon that the double-stranded AAV vector.

Example 2. Evalutation of ss-AAV containing opti-hu-FKRP-CpG(-) and ds-AAV containing opti-hu-FKRP gene

In addition, we evaluated the advantage of using codon-optimized plus CpG-depleted hu-FKRP codon (FKRP-CpG(-)) to determine if therapeutic effects could be further improved by utilizing less immunogenic CpG-depleted FKRP coding sequences.

The human codon-optimized and CpG depleted FKRP gene (**SEQ ID NO:1**) was synthesized from GeneArt. Then the opti-hu-FKRP-CpG(-) gene was cloned into ss-AAV vector plasmids driven by different promoters: muscle specific promoter CK, muscle specific promoter syn100, and ubiquitous CB promoter (chicken beta actin promoter with a cytomegalovirus early gene enhancer). The expression of FKRP was evaluated *in vitro* by transfection of the plasmids into HEK (human embryonic kidney) cells 293. As revealed in **FIG. 3**, the expression level of opti-hu-FKRP-CpG(-) plasmid was similar to the original plasmid (opti-hu-FKRP) which contains CpG motifs.

To evaluate the therapeutic effects offered by different AAV cassettes, we injected AAV9 vector comprising the different FKRP constructs driven by different promoters into 6 to 8 weeks-old *E310^{stop}/L276I* mice via tail vein injection at the dosage of 6 x 10^{13}vg/kg (n = 8 for each group). At two weeks post injection, the treated and control mice were subjected to bleeding and the serum were subjected to a set of cytokines testing including IL1, IL4, IL6, IL10, IL17, TNF-alpha, TGF-beta, and IFN-gamma. In general, we did not observe significant difference in serum cytokine levels between treated and control groups, however, we did observe a subtle difference of serum IL17A concentration (**FIG. 4**). The serum IL17A level was slightly decreased in the FKRP-CpG(-) group as compared to the FKRP group, indicating safer immune profile of the FKRP-CpG(-) coding sequences. As a therapeutic readout, the serum CK was measured at 6 weeks post injection.

Example 3. Incorporation of FKRP-CpG(-) into ds-AAV vector and evaluating their therapeutic effects in vivo

Lastly, we evaluated the therapeutic effects of double-stranded AAV vectors containing FKRP-CpG(-) *in vivo*.

To compare the effects of the FKRP-CpG-depleted sequences (e.g., **SEQ ID NO:1**, **SEQ ID NO:6**) in ds-AAV vectors, we created it with the FKRP construct containing CpG (e.g., **SEQ ID NO:7**, **SEQ ID NO:8**) (**FIG. 5**). These constructs were packaged into AAV9 vectors, and the vectors were delivered into 6 to 7 weeks old homozygous L276I FKRP-mutant mice via tail vein injection. We treated males and females with slightly different vector dosages (n = 7) for each gender with $5x10^{13}$ vg/kg for males and $3x10^{13}$ vg/kg for females. The serum CK level was measured at 6 weeks post injection. Significant therapeutic responses were observed in both male and females at 6 weeks post treatment. In particular, in the female group, with the exception of one outlier, the treated mice displayed significant reduction in serum CK level. However, in the male group, we noticed significant reduction in serum CK level in the two CpG-depleted groups, with moderate reduction in serum CK level in the two opti-hu-FKRP groups in 6-week and 12-week post vector injection (**FIG. 6**). The data indicates that ds-AAV vector containing CpG-depleted FKRP gene driven by muscle-specific promoters Syn100 demonstrated superior therapeutic effects *in vivo*.

It will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present invention. Therefore, it should be clearly understood that the forms of the present invention are illustrative only and are not intended to limit the scope of the present invention.

All publications, patent applications, patents, patent publications, sequences identified by GenBank® database accession numbers and other references cited herein are incorporated by reference in their entireties for the teachings relevant to the sentence and/or paragraph in which the reference is presented.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

SEQUENCES

Artificial sequence opti-hu-FKRP-CpG(-)

ATGAGACTGACAAGATGTCAAGCTGCCCTGGCTGCTGCCATCACACTGAATCTGC
TGGTGCTGTTCTATGTGTCCTGGCTGCAGCATCAGCCCAGAAACTCTAGAGCCAG
AGGACCCAGAAGGGCCTCTGCTGCTGGACCTAGAGTGACAGTGCTTGTCAGAGA
GTTTGAGGCCTTTGACAATGCTGTGCCTGAGCTGGTGGACAGCTTCCTGCAGCAA
GATCCTGCTCAGCCTGTGGTGGTGGCTGCTGATACACTGCCTTATCCTCCACTGG
CTCTGCCTAGAATCCCCAATGTTAGACTGGCCCTCCTGCAGCCTGCTCTGGATAG
ACCTGCTGCTGCTTCCAGACCTGAGACATATGTGGCCACTGAGTTTGTGGCCCTG
GTGCCTGATGGTGCCAGAGCTGAAGCTCCTGGCCTGCTGGAAAGAATGGTTGAG
GCCCTGAGAGCTGGATCTGCCAGACTGGTTGCTGCTCCTGTGGCTACAGCCAATC
CTGCCAGATGTCTGGCCCTGAATGTGTCCCTGAGAGAATGGACAGCCAGATATG
GTGCTGCCCCAGCTGCTCCTAGATGTGATGCTCTTGATGGGGATGCTGTGGTCCT
GCTGAGAGCCAGGGATCTGTTCAATCTGTCTGCCCCACTGGCCAGACCTGTGGGC
ACATCTCTGTTTCTGCAGACAGCTCTGAGAGGCTGGGCTGTGCAGCTGCTGGATC
TCACCTTTGCTGCTGCAAGACAGCCTCCTCTGGCCACAGCTCATGCCAGATGGAA
GGCTGAGAGAGAGGGCAGAGCTAGAAGGGCTGCTCTGCTCAGAGCACTGGGCAT
CAGACTGGTGTCTTGGGAAGGTGGCAGACTTGAGTGGTTTGGCTGCAACAAAGA
AACCACCAGATGCTTTGGCACAGTTGTGGGAGACACCCCTGCCTACCTGTATGAG
GAAAGATGGACCCCACCTTGCTGTCTGAGAGCCCTGAGGGAAACAGCTAGATAT
GTTGTTGGAGTGCTTGAGGCTGCTGGTGTCAGATACTGGCTGGAAGGTGGAAGTC
TGCTGGGAGCTGCTAGGCATGGGGACATCATCCCTTGGGACTATGATGTGGACCT
GGGCATCTACCTGGAAGATGTGGGCAATTGTGAACAGCTGAGAGGGGCTGAAGC
TGGCTCTGTTGTGGATGAGAGAGGCTTTGTCTGGGAGAAAGCTGTTGAGGGAGA
CTTCTTCAGAGTGCAGTACTCTGAGAGCAACCACCTCCATGTGGATCTGTGGCCA
TTCTACCCCAGAAATGGGGTCATGACAAAGGACACCTGGCTGGACCACAGACAG
GATGTGGAATTCCCTGAGCACTTTCTGCAGCCTCTGGTGCCACTGCCTTTTGCTGG
ATTTGTGGCTCAGGCCCCTAACAACTACAGAAGATTCCTGGAACTGAAGTTTGGC
CCTGGGGTCATAGAGAACCCTCAGTACCCTAATCCTGCACTGCTGAGCCTGACTG
GATCTGGCTGATGA **SEQ ID NO:1**

Synthetic promoter (Syn100)

Cggccgtccgccctcggcaccattcctcacgacaccgaaatatggcgacgggtgaggaatggtggggagttatttttagagcggtga
ggaatggtgggcaggcagcaggtgttgggggagttatttttagagcggggagttatttttagagcggtgaggaatggtggacaccgaa
atatggcgacgggtgaggaatggtgccgtcgccatatttgggtgtcccgtccgccctcggccggggccgcattcctgggggccggg
cggtgctcccgcccgcctcgataaaaggctccggggccggcggcggcccacgagctacccggaggagcgggaggcgtctctgcc
agcggtccgacgcgcagtcagcaccaggtaggtgggcaccgcgccgtgccgtgcc **SEQ ID NO:2**

Homo sapiens Vh4-Ig-intron3

gtgagtatctcagggatccagacatggggatatgggaggtgcctctgatcccagggctcactgtgggtctctctgttcacag **SEQ ID NO:3**

Artificial sequence Poly A

tagagctcgctgatcagcctcgactgtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtg
ccactcccactgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggca
ggacagcaagggggaggattgggaagacaatagcaggcatgctggggag **SEQ ID NO:4**

Artificial sequence

aggcctaataaagagctcagatgcatcgatcagagtgtgttggtttttgtgtg **SEQ ID NO:5**

Artificial sequence opti-hu-FKRP-CpG(-) with poly A tail

ATGAGACTGACAAGATGTCAAGCTGCCCTGGCTGCTGCCATCACACTGAATCTGC
TGGTGCTGTTCTATGTGTCCTGGCTGCAGCATCAGCCCAGAAACTCTAGAGCCAG
AGGACCCAGAAGGGCCTCTGCTGCTGGACCTAGAGTGACAGTGCTTGTCAGAGA
GTTTGAGGCCTTTGACAATGCTGTGCCTGAGCTGGTGGACAGCTTCCTGCAGCAA
GATCCTGCTCAGCCTGTGGTGGTGGCTGCTGATACACTGCCTTATCCTCCACTGG
CTCTGCCTAGAATCCCCAATGTTAGACTGGCCCTCCTGCAGCCTGCTCTGGATAG
ACCTGCTGCTGCTTCCAGACCTGAGACATATGTGGCCACTGAGTTTGTGGCCCTG
GTGCCTGATGGTGCCAGAGCTGAAGCTCCTGGCCTGCTGGAAAGAATGGTTGAG
GCCCTGAGAGCTGGATCTGCCAGACTGGTTGCTGCTCCTGTGGCTACAGCCAATC
CTGCCAGATGTCTGGCCCTGAATGTGTCCCTGAGAGAATGGACAGCCAGATATG
GTGCTGCCCCAGCTGCTCCTAGATGTGATGCTCTTGATGGGGATGCTGTGGTCCT
GCTGAGAGCCAGGGATCTGTTCAATCTGTCTGCCCCACTGGCCAGACCTGTGGGC
ACATCTCTGTTTCTGCAGACAGCTCTGAGAGGCTGGGCTGTGCAGCTGCTGGATC
TCACCTTTGCTGCTGCAAGACAGCCTCCTCTGGCCACAGCTCATGCCAGATGGAA
GGCTGAGAGAGAGGGCAGAGCTAGAAGGGCTGCTCTGCTCAGAGCACTGGGCAT
CAGACTGGTGTCTTGGGAAGGTGGCAGACTTGAGTGGTTTGGCTGCAACAAAGA
AACCACCAGATGCTTTGGCACAGTTGTGGGAGACACCCCTGCCTACCTGTATGAG
GAAAGATGGACCCCACCTTGCTGTCTGAGAGCCCTGAGGGAAACAGCTAGATAT
GTTGTTGGAGTGCTTGAGGCTGCTGGTGTCAGATACTGGCTGGAAGGTGGAAGTC
TGCTGGGAGCTGCTAGGCATGGGGACATCATCCCTTGGGACTATGATGTGGACCT
GGGCATCTACCTGGAAGATGTGGGCAATTGTGAACAGCTGAGAGGGGCTGAAGC
TGGCTCTGTTGTGGATGAGAGAGGCTTTGTCTGGGAGAAAGCTGTTGAGGGAGA
CTTCTTCAGAGTGCAGTACTCTGAGAGCAACCACCTCCATGTGGATCTGTGGCCA
TTCTACCCCAGAAATGGGGTCATGACAAAGGACACCTGGCTGGACCACAGACAG
GATGTGGAATTCCCTGAGCACTTTCTGCAGCCTCTGGTGCCACTGCCTTTTGCTGG
ATTTGTGGCTCAGGCCCCTAACAACTACAGAAGATTCCTGGAACTGAAGTTTGGC
CCTGGGGTCATAGAGAACCCTCAGTACCCTAATCCTGCACTGCTGAGCCTGACTG
GATCTGGCTGATGA***aggcctaataaagagctcagatgcatcgatcagagtgtgttggttttttgtgtg*** **SEQ ID NO:6**

Artificial sequence opti-hu-FKRP expression construct

cggccgtccgccctcggcaccattcctcacgacaccgaaatatggcgacgggtgaggaatggtggggagttatttttagagcggt
gaggaatggtgggcaggcagcaggtgttgggggagttatttttagagcggggagttatttttagagcggtgaggaatggtggaca
ccgaaatatggcgacgggtgaggaatggtgccgtcgccatatttgggtgtcccgtccgccctcggccggggccgcattcctgggg
gccgggcggtgctcccgcccgcctcgataaaaggctccggggccggcggcggcccacgagctacccggaggagcgggaggc
*gtctctgccagcggtccgacgcgcagtcagcaccaggtaggtgggcaccgcgccgtgccgtgcc***gtgagtatctcagggatcca**
gacatggggatatgggaggtgcctctgatcccagggctcactgtgggtctctctgttcacagATGAGACTGACAAG
ATGTCAAGCTGCCCTGGCTGCTGCCATCACACTGAATCTGCTGGTGCTGTTCTAT
GTGTCCTGGCTGCAGCATCAGCCCAGAAACTCTAGAGCCAGAGGACCCAGAAGG
GCCTCTGCTGCTGGACCTAGAGTGACAGTGCTTGTCAGAGAGTTTGAGGCCTTTG
ACAATGCTGTGCCTGAGCTGGTGGACAGCTTCCTGCAGCAAGATCCTGCTCAGCC
TGTGGTGGTGGCTGCTGATACACTGCCTTATCCTCCACTGGCTCTGCCTAGAATCC
CCAATGTTAGACTGGCCCTCCTGCAGCCTGCTCTGGATAGACCTGCTGCTGCTTC
CAGACCTGAGACATATGTGGCCACTGAGTTTGTGGCCCTGGTGCCTGATGGTGCC
AGAGCTGAAGCTCCTGGCCTGCTGGAAAGAATGGTTGAGGCCCTGAGAGCTGGA
TCTGCCAGACTGGTTGCTGCTCCTGTGGCTACAGCCAATCCTGCCAGATGTCTGG
CCCTGAATGTGTCCCTGAGAGAATGGACAGCCAGATATGGTGCTGCCCCAGCTGC
TCCTAGATGTGATGCTCTTGATGGGGATGCTGTGGTCCTGCTGAGAGCCAGGGAT
CTGTTCAATCTGTCTGCCCCACTGGCCAGACCTGTGGGCACATCTCTGTTTCTGCA

GACAGCTCTGAGAGGCTGGGCTGTGCAGCTGCTGGATCTCACCTTTGCTGCTGCA
AGACAGCCTCCTCTGGCCACAGCTCATGCCAGATGGAAGGCTGAGAGAGAGGGC
AGAGCTAGAAGGGCTGCTCTGCTCAGAGCACTGGGCATCAGACTGGTGTCTTGG
GAAGGTGGCAGACTTGAGTGGTTTGGCTGCAACAAAGAAACCACCAGATGCTTT
GGCACAGTTGTGGGAGACACCCCTGCCTACCTGTATGAGGAAAGATGGACCCCA
CCTTGCTGTCTGAGAGCCCTGAGGGAAACAGCTAGATATGTTGTTGGAGTGCTTG
AGGCTGCTGGTGTCAGATACTGGCTGGAAGGTGGAAGTCTGCTGGGAGCTGCTA
GGCATGGGGACATCATCCCTTGGGACTATGATGTGGACCTGGGCATCTACCTGGA
AGATGTGGGCAATTGTGAACAGCTGAGAGGGGCTGAAGCTGGCTCTGTTGTGGA
TGAGAGAGGCTTTGTCTGGGAGAAAGCTGTTGAGGGAGACTTCTTCAGAGTGCA
GTACTCTGAGAGCAACCACCTCCATGTGGATCTGTGGCCATTCTACCCCAGAAAT
GGGGTCATGACAAAGGACACCTGGCTGGACCACAGACAGGATGTGGAATTCCCT
GAGCACTTTCTGCAGCCTCTGGTGCCACTGCCTTTTGCTGGATTTGTGGCTCAGGC
CCCTAACAACTACAGAAGATTCCTGGAACTGAAGTTTGGCCCTGGGGTCATAGA
GAACCCTCAGTACCCTAATCCTGCACTGCTGAGCCTGACTGGATCTGGCTGATGA
aggcctaataaagagctcagatgcatcgatcagagtgtgttggttttttgtgtg **SEQ ID NO:7**

Artificial sequence opti-huFKRP
ATGAGACTGACAAGATGCCAGGCCGCCCTGGCCGCTGCCATCACACTGAATCTG
CTGGTGCTGTTCTATGTGTCCTGGCTGCAGCACCAGCCCCGGAACTCTAGAGCCA
GAGGCCCAAGAAGGGCCTCTGCCGCCGGACCTAGAGTGACAGTGCTCGTGCGCG
AGTTCGAGGCCTTCGACAATGCCGTGCCCGAGCTGGTGGACAGCTTCCTGCAGCA
AGACCCTGCTCAGCCTGTGGTGGTGGCCGCCGATACACTGCCTTATCCTCCACTG
GCCCTGCCCAGAATCCCCAATGTGCGACTGGCTCTGCTGCAGCCCGCCCTGGATA
GACCTGCCGCCGCTAGCAGACCTGAGACATACGTGGCCACCGAGTTCGTGGCCCT
GGTGCCTGATGGCGCCAGAGCTGAAGCTCCCGGCCTGCTGGAAAGAATGGTGGA
AGCCCTGAGAGCCGGCAGCGCCAGACTGGTGGCTGCTCCTGTGGCTACCGCCAA
CCCTGCCAGATGTCTGGCCCTGAATGTGTCCCTGAGAGAGTGGACCGCCAGATAC
GGCGCTGCCCCTGCCGCTCCTAGATGTGATGCTCTGGATGGCGACGCCGTGGTGC
TGCTGAGAGCCAGGGACCTGTTCAACCTGAGCGCCCCTCTGGCCAGACCTGTGGG
CACAAGCCTGTTTCTGCAGACAGCCCTGAGGGGCTGGGCCGTGCAGCTGCTGGAT
CTGACATTTGCCGCTGCCAGACAGCCTCCTCTGGCCACAGCCCATGCCAGATGGA
AGGCCGAGAGAGAGGGCAGAGCCAGAAGGGCTGCTCTGCTGAGGGCCCTGGGC
ATCAGACTGGTGTCTTGGGAAGGCGGCAGACTCGAGTGGTTCGGCTGCAACAAA
GAAACCACCCGGTGCTTCGGCACCGTCGTGGGCGATACACCAGCCTACCTGTACG
AGGAAAGATGGACCCCCCCTTGCTGCCTGCGGGCCCTGAGAGAAACAGCCAGAT
ATGTCGTGGGCGTGCTGGAAGCCGCTGGCGTGCGATATTGGCTGGAAGGCGGAT
CTCTGCTGGGAGCCGCCAGGCACGGCGACATCATCCCTTGGGACTACGACGTGG
ACCTGGGCATCTACCTGGAAGATGTGGGCAACTGCGAGCAGCTGAGAGGCGCCG
AAGCCGGCTCTGTGGTGGATGAGAGGGGCTTCGTGTGGGAGAAGGCCGTGGAAG
GCGACTTCTTCCGGGTGCAGTACAGCGAGAGCAACCATCTGCATGTGGACCTGTG
GCCCTTCTACCCCCGGAACGGCGTGATGACCAAGGACACCTGGCTGGACCACCG
GCAGGACGTGGAATTCCCCGAGCACTTTCTGCAGCCCCTGGTGCCACTGCCTTTC
GCCGGATTTGTGGCCCAGGCCCCCAACAACTACCGGCGGTTCCTGGAACTGAAGT
TCGGCCCTGGCGTGATCGAGAACCCCCAGTACCCTAACCCTGCCCTGCTGAGCCT
GACCGGCAGCGGCTAATGA **SEQ ID NO:8**

Gallus gallus

gcggcggcccacgagctacccggaggagcgggaggcgtctctgccagcggtccgacgcgcagtcagcaccaggtaggtgggca ccgcgccgtgccgtgcc **SEQ ID NO:9**

Native nucleotide sequence encoding human FKRP: (1488 nucleotides)

ATG CGG CTC ACC CGC TGC CAG GCT GCC CTG GCG GCC GCC ATC ACC CTC AAC CTT CTG GTC CTC TTC TAT GTC TCG TGG CTG CAG CAC CAG CCT AGG AAT TCC CGG GCC CGG GGG CCC CGT CGT GCC TCT GCT GCC GGC CCC CGT GTC ACC GTC CTG GTG CGG GAG TTC GAG GCA TTT GAC AAC GCG GTG CCC GAG CTG GTA GAC TCC TTC CTG CAG CAA GAC CCA GCC CAG CCC GTG GTG GTG GCA GCC GAC ACG CTC CCC TAC CCG CCC CTG GCC CTG CCC CGC ATC CCC AAC GTG CGT CTG GCG CTG CTC CAG CCC GCC CTG GAC CGG CCA GCC GCA GCC TCG CGC CCG GAG ACC TAC GTG GCC ACC GAG TTT GTG GCC CTA GTA CCT GAT GGG GCG CGG GCT GAG GCA CCT GGC CTG CTG GAG CGC ATG GTG GAG GCG CTC CGC GCA GGA AGC GCA CGT CTG GTG GCC GCC CCG GTT GCC ACG GCC AAC CCT GCC AGG TGC CTG GCC CTG AAC GTC AGC CTG CGA GAG TGG ACC GCC CGC TAT GGC GCA GCC CCC GCC GCG CCC CGC TGC GAC GCC CTG GAC GGA GAT GCT GTG GTG CTC CTG CGC GCC CGC GAC CTC TTC AAC CTC TCG GCG CCC CTG GCC CGG CCG GTG GGC ACC AGC CTC TTT CTG CAG ACC GCC CTT CGC GGC TGG GCG GTG CAG CTG CTG GAC TTG ACC TTC GCC GCG GCG CGC CAG CCC CCG CTG GCC ACG GCC CAC GCG CGC TGG AAG GCT GAG CGC GAG GGA CGC GCT CGG CGG GCG GCG CTG CTC CGC GCG CTG GGC ATC CGC CTA GTG AGC TGG GAA GGC GGG CGG CTG GAG TGG TTC GGC TGC AAC AAG GAG ACC ACG CGC TGC TTC GGA ACC GTG GTG GGC GAC ACG CCC GCC TAC CTC TAC GAG GAG CGC TGG ACG CCC CCC TGC TGC CTG CGC GCG CTG CGC GAG ACC GCC CGC TAT GTG GTG GGC GTG CTG GAG GCT GCG GGC GTG CGC TAC TGG CTC GAG GGC GGC TCA CTG CTG GGG GCC GCC CGC CAC GGG GAC ATC ATC CCA TGG GAC TAC GAC GTG GAC CTG GGC ATC TAC TTG GAG GAC GTG GGC AAC TGC GAG CAG CTG CGG GGG GCA GAG GCC GGC TCG GTG GTG GAT GAG CGC GGC TTC GTA TGG GAG AAG GCG GTC GAG GGC GAC TTT TTC CGC GTG CAG TAC AGC GAA AGC AAC CAC TTG CAC GTG GAC CTG TGG CCC TTC TAC CCC CGC AAT GGC GTC ATG ACC AAG GAC ACG TGG CTG GAC CAC CGG CAG GAT GTG GAG TTT CCC GAG CAC TTC CTG CAG CCG CTG GTG CCC CTG CCC TTT GCC GGC TTC GTG GCG CAG GCG CCT AAC AAC TAC CGC CGC TTC CTG GAG CTC AAG TTC GGG CCC GGG GTC ATC GAG AAC CCC CAG TAC CCC AAC CCG GCA CTG CTG AGT CTG ACG GGA AGC GGC TGA(**SEQ ID NO:10**)

What is claimed is:

1. A synthetic polynucleotide encoding a human fukutin-related protein (FKRP), wherein the synthetic polynucleotide comprises:

the nucleotide sequence of **SEQ ID NO:1**; and/or

a nucleotide sequence having at least 90% identity to **SEQ ID NO:1**.

2. The synthetic polynucleotide of claim 1, wherein the synthetic polynucleotide is operably linked to a promoter.

3. The synthetic polynucleotide of claim 2, wherein the promoter is a muscle specific promoter.

4. The synthetic polypeptide of claim 2 or claim 3, wherein the promoter is a synthetic muscle specific promoter.

5. The synthetic polypeptide of claim 4, wherein the synthetic muscle specific promoter comprises a polynucleotide sequence having at least 90% identity to the nucleotide sequence of **SEQ ID NO:2**.

6. The synthetic polynucleotide of claim 5, further comprising an enhancer sequence.

7. The synthetic polynucleotide of claim 6, wherein the enhancer sequence is an intron.

8. The synthetic polynucleotide of claim 7, wherein the intron is a Vh4-Ig-intron3 comprising the nucleotide sequence of **SEQ ID NO:3**.

9. The synthetic polynucleotide of any one of claims 1 to 8, further comprising a poly A tail.

10. The synthetic polynucleotide of claim 9, wherein the poly A tail is from bovine growth hormone.

11. The synthetic polynucleotide of claim 9 or claim 10, wherein the poly A tail comprises the nucleotide sequence of **SEQ ID NO:4**.

12. The synthetic polynucleotide of claim 9, wherein the poly A tail comprises the nucleotide sequence of **SEQ ID NO:5**.

13. The synthetic polynucleotide of claim 12, wherein the synthetic polynucleotide comprises the nucleotide sequence of **SEQ ID NO:6**; and/or a nucleotide sequence having at least 80% identity to the nucleotide sequence of **SEQ ID NO:6**.

14. A synthetic polynucleotide encoding a human fukutin-related protein (FKRP), wherein the synthetic polynucleotide comprises the nucleotide sequence of **SEQ ID NO:6** and/or a nucleotide sequence having at least 80% identity to the nucleotide sequence of **SEQ ID NO:6**.

15. A synthetic polynucleotide encoding a human fukutin-related protein (FKRP), wherein the synthetic polynucleotide comprises the nucleotide sequence of **SEQ ID NO:7** and/or a nucleotide sequence having at least 80% identity to the nucleotide sequence of **SEQ ID NO:7**.

16. A vector comprising the synthetic polynucleotide of any one of claims 1 to 15.

17. The vector of claim 16, wherein the vector is a viral vector.

18. The vector of claim 17, wherein the viral vector is single stranded.

19. The vector of claim 17, wherein the viral vector is double stranded.

20. The vector of claim 19, wherein the viral vector comprises a mutated terminal repeat (ITR).

21. The vector of any one of claims 15 to 20, further comprising a mir122 binding element.

22. The vector of any one of claims 15 to 21, wherein the vector is an adeno-associated virus (AAV) vector.

23. The vector of claim 22, wherein the AAV vector is an AAV type 1, AAV type 2, AAV type 3a, AAV type 3B, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type 10, AAV type 11, avian AAV, bovine AAV, canine AAV, equine AAV, or ovine AAV.

24. The vector of claim 22 or claim 23, wherein the AAV vector is an AAV9 vector.

25. A transformed cell comprising the synthetic polynucleotide of any one of claims 1 to 15 and/or the vector of any one of claims 16 to 24.

26. A transgenic animal comprising the synthetic polynucleotide of any one of claims 1 to 15, the vector of any one of claims 16 to 24, and/or the transformed cell of claim 24.

27. A method of delivering a nucleic acid to a cell comprising delivering to the cell a therapeutically effective amount of the synthetic polynucleotide of any one of claims 1 to 15, and/or the vector of any one of claims 16 to 24.

28. A method of delivering a nucleic acid to a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of the synthetic polynucleotide of any one of claims 1 to 15, the vector of any one of claims 16 to 24, and/or the transformed cell of claim 25.

29. A method of treating a dystroglycanopathy in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of the synthetic polynucleotide of any one of claims 1 to 15, the vector of any one of claims 16 to 24, and/or the transformed cell of claim 25, thereby treating dystroglycanopathy in the subject.

30. The method of claim 29, wherein the dystroglycanopathy comprises a mutation in the nucleic acid encoding FKRP and/or a deficiency in glycosylation of alpha-dystroglycan (α-DG), or any combination thereof.

31. The method of claim 29 or claim 30, wherein the dystroglycanopathy is limb girdle muscular dystrophy 2I, congenital muscular dystrophy, Walker-Warburg syndrome, muscle-eye-brain disease, or any combination thereof.

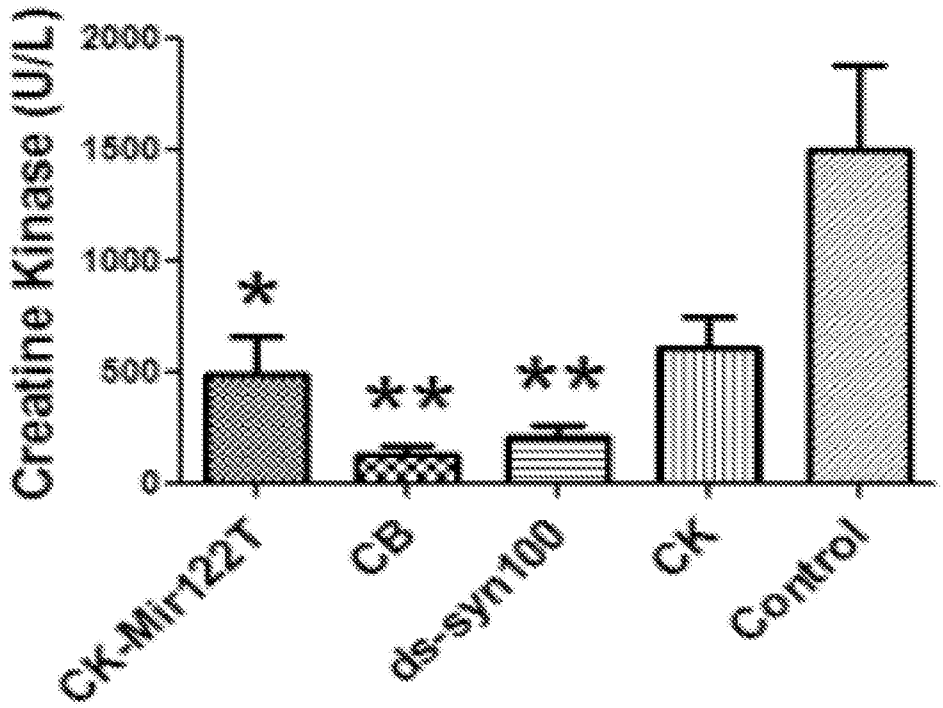
32. A method of reducing serum creatine kinase levels in a subject in need thereof, comprising delivering to the subject the synthetic polynucleotide of any one of claims 1 to 15 and/or the vector of any one of claims 16 to 24, and/or the transformed cell of claim 25, wherein the synthetic polynucleotide is expressed in the subject, thereby producing human FKRP and reducing serum creatine kinase levels in the subject.

33. A method of reducing an immune response when administering an FKRP expressing vector, comprising delivering to the subject the synthetic polynucleotide of any one of claims 1 to 15 and/or the vector of any one of claims 16 to 24, and/or the transformed cell of claim 25, wherein the synthetic polynucleotide is expressed in the subject, thereby reducing the immune response when compared to administering a codon optimized human FKRP expressing vector (e.g., encoding the nucleotide sequence of **SEQ ID NO:8**).

34. The method of claim 33, wherein reducing an immune response comprises reduced IL17A levels as compared to a subject having been administered a vector expressing an optimized human FKRP having the nucleotide sequence of **SEQ ID NO:8**.

FIG. 1

A


Creatine Kinase (U/L)
2000
1500
1000
500
0
*
**
**
CK-Mir122T
CB
ds-syn100
CK
Control

B

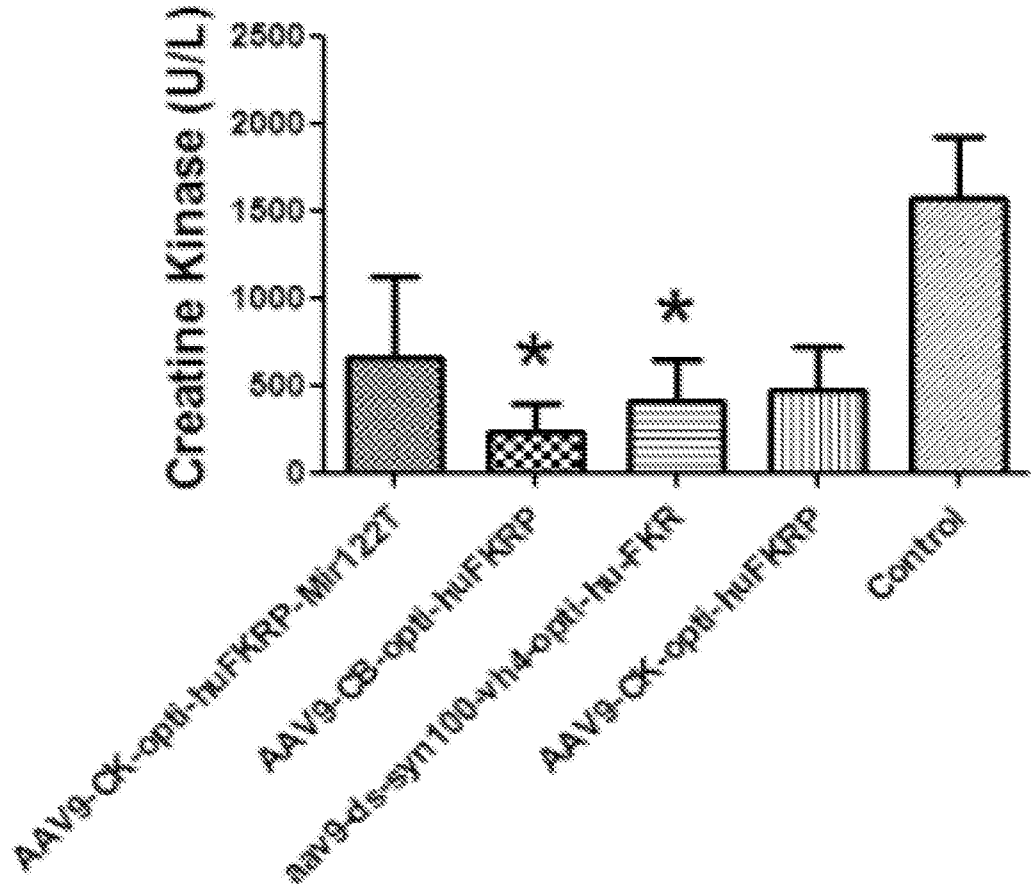

Creatine Kinase (U/L)
2500
2000
1500
1000
500
0
*
*
AAV9-CK-opti-huFKRP-Mir122T
AAV9-CB-opti-huFKRP
aav9-ds-syn100-vh4-opti-hu-FKR
AAV9-CK-opti-huFKRP
Control

FIG. 1 (con't)
C

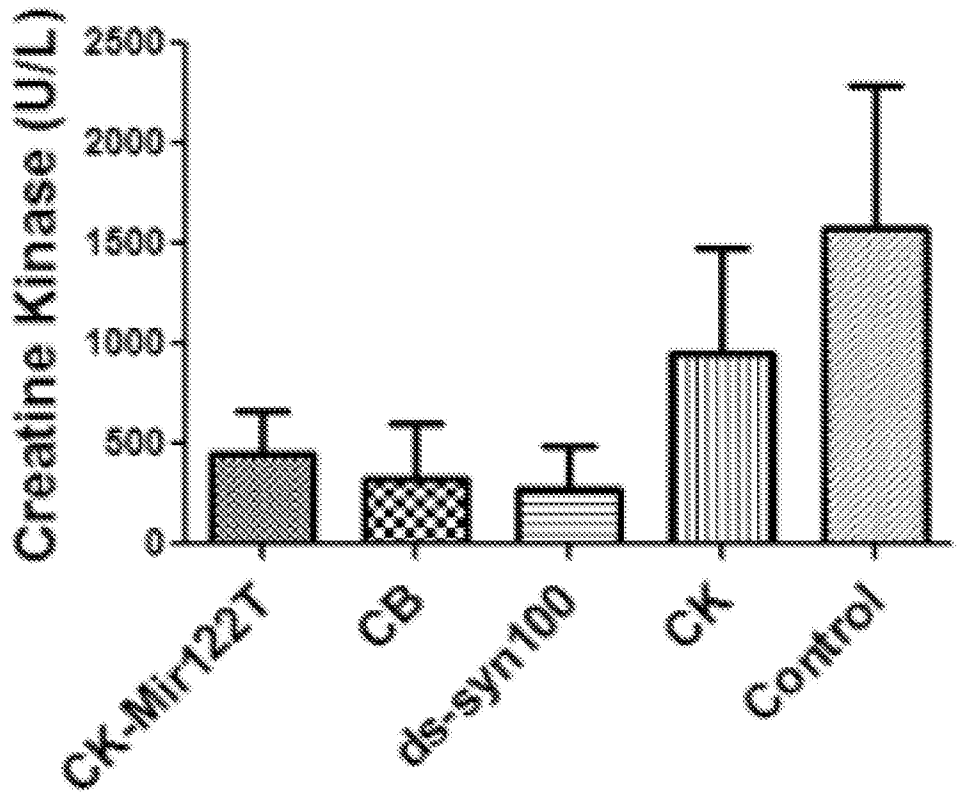
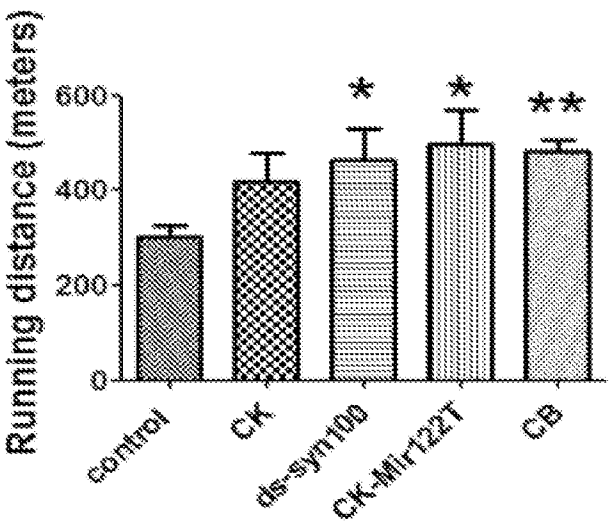


FIG. 2



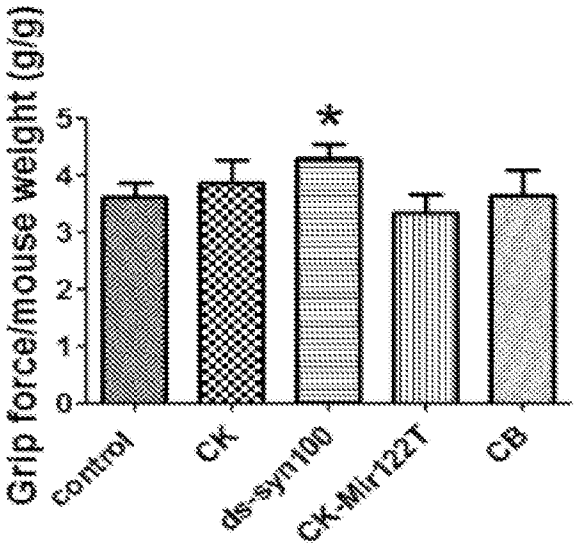


FIG. 3

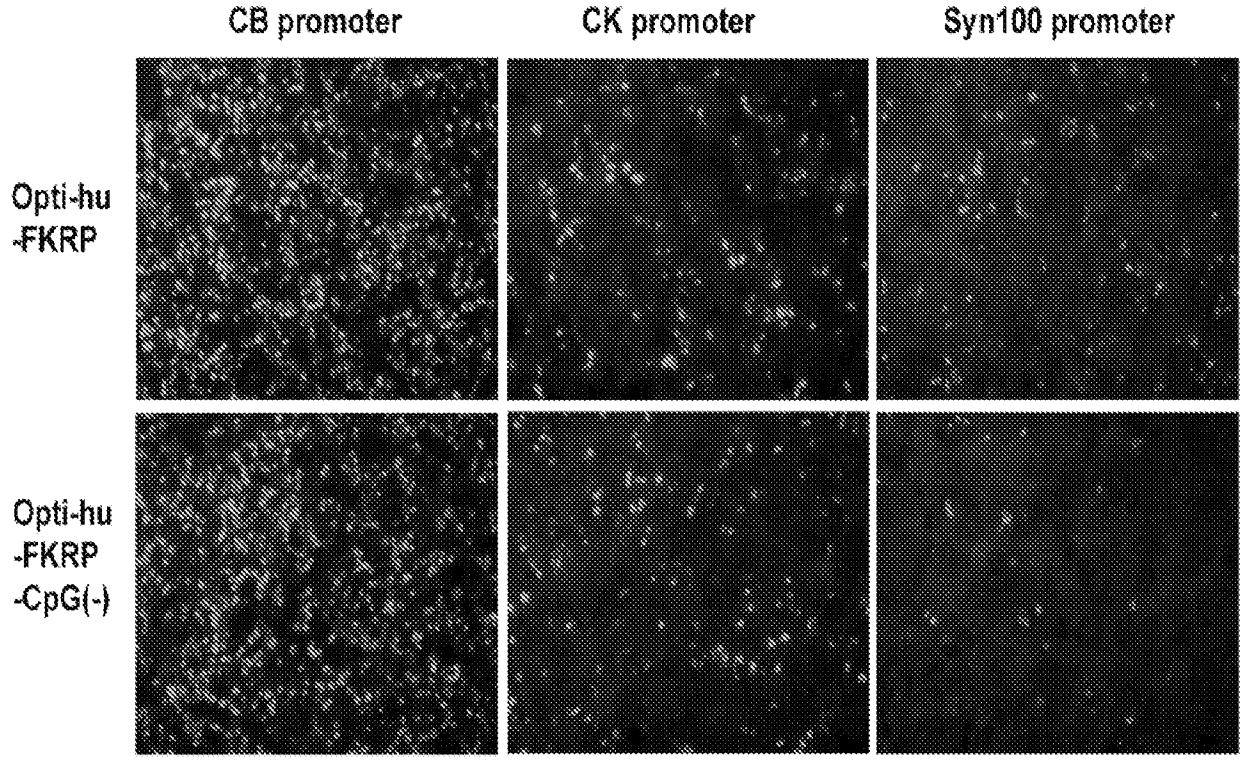

CB promoter
CK promoter
Syn100 promoter
Opti-hu -FKRP
Opti-hu -FKRP -CpG(-)

FIG. 4

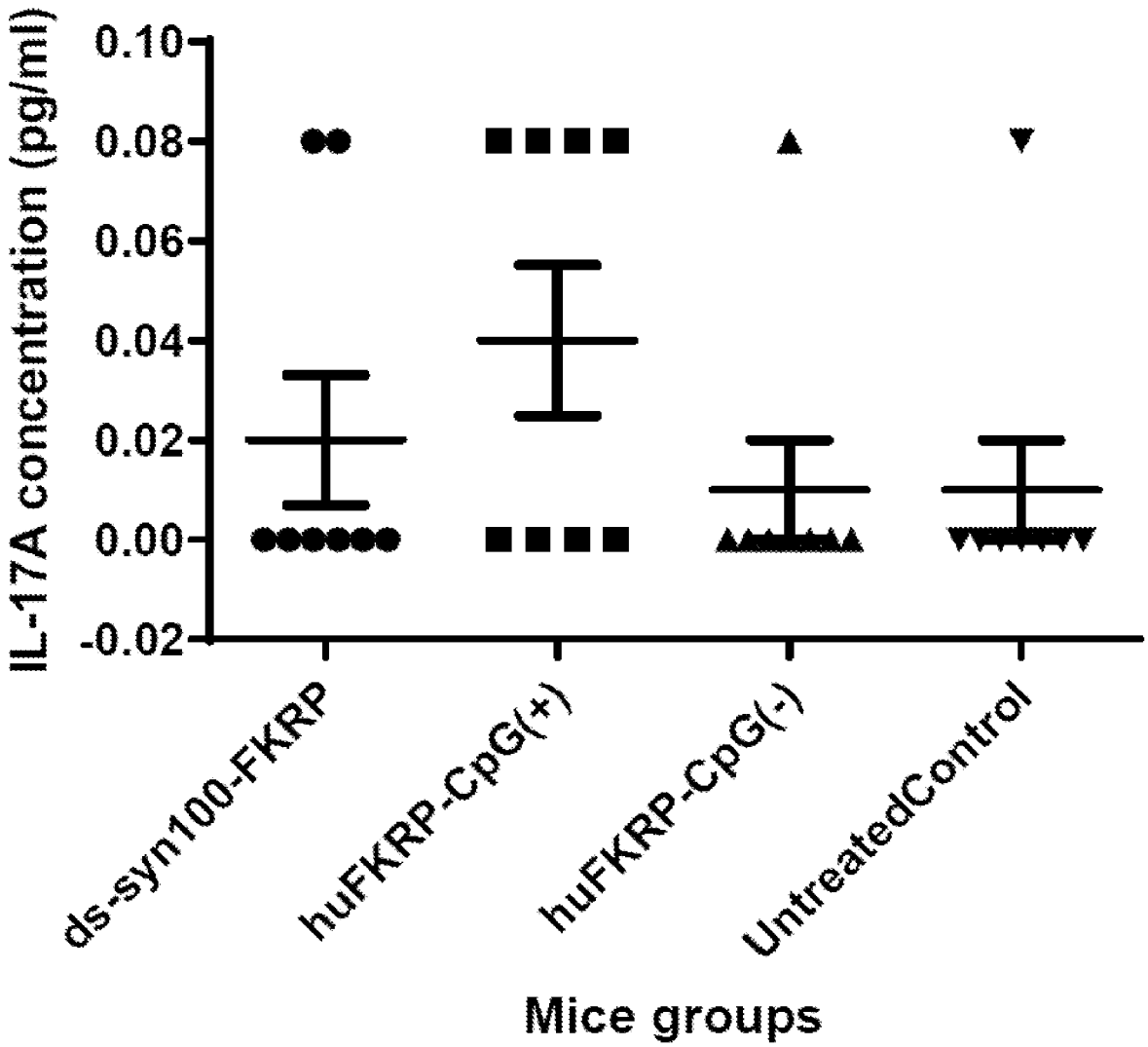

IL-17A concentration (pg/ml)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
ds-syn100-FKRP
huFKRP-CpG(+)
huFKRP-CpG(-)
UntreatedControl
Mice groups

FIG. 5

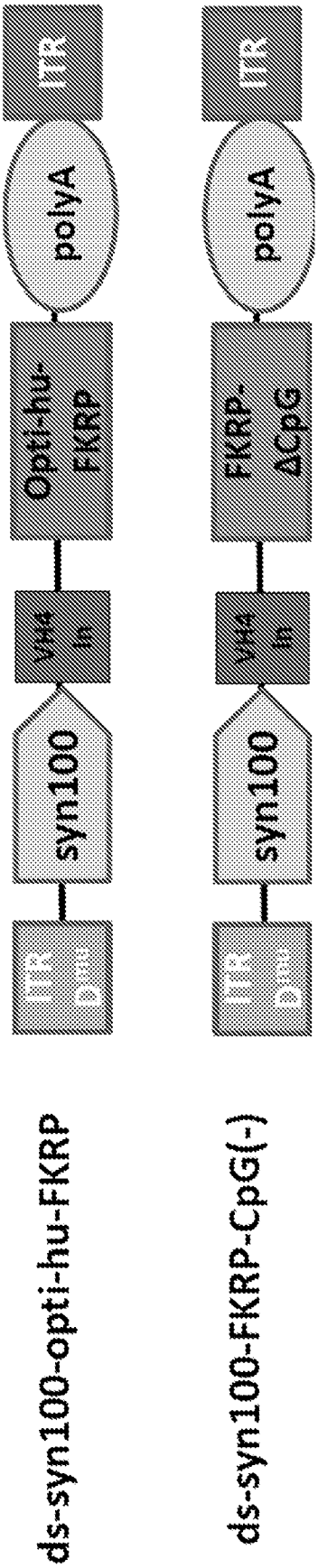

ITR
polyA
Opti-hu-FKRP
VH4 In
syn100
ITR
ds-syn100-opti-hu-FKRP
ITR
polyA
FKRP-ΔCpG
VH4 In
syn100
ITR
ds-syn100-FKRP-CpG(-)

FIG. 6
A

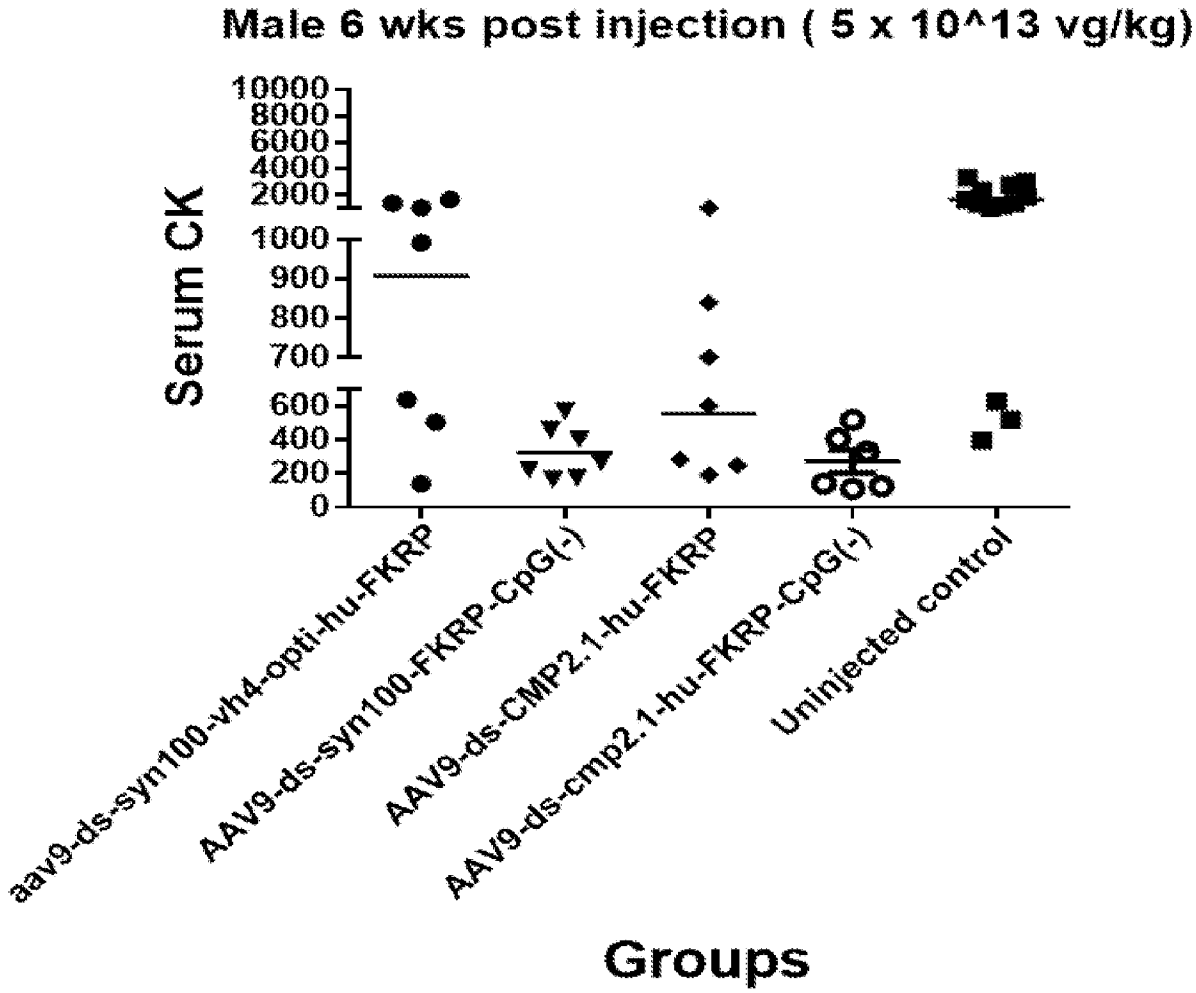

Male 6 wks post injection (5 x 10^13 vg/kg)
Serum CK
10000
8000
6000
4000
2000
1000
900
800
700
600
400
200
0
aav9-ds-syn100-vh4-opti-hu-FKRP
AAV9-ds-syn100-FKRP-CpG(-)
AAV9-ds-CMP2.1-hu-FKRP
AAV9-ds-cmp2.1-hu-FKRP-CpG(-)
Uninjected control
Groups

FIG. 6 (con't)
B

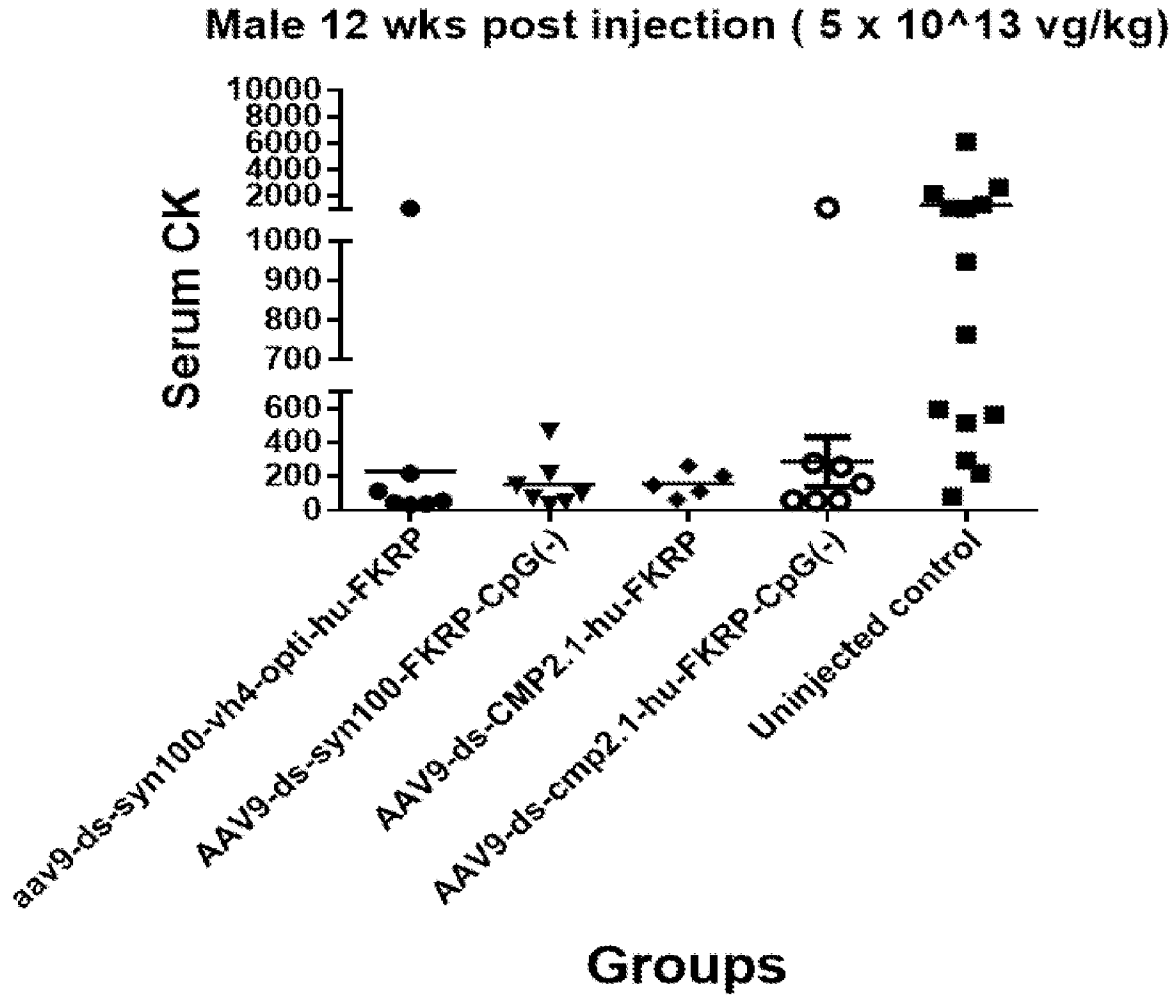
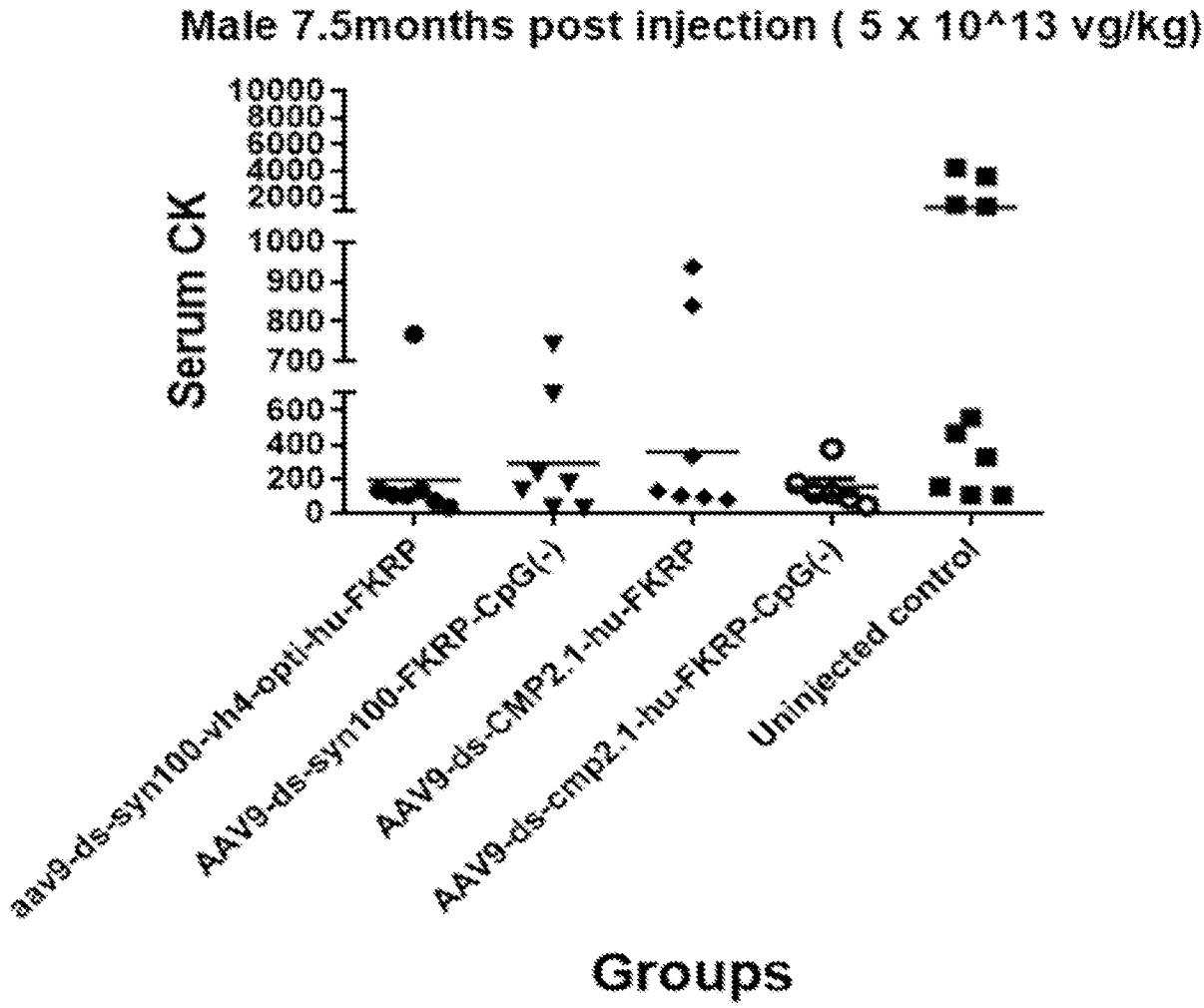

Male 12 wks post injection (5 x 10^13 vg/kg)
Serum CK
10000
8000
6000
4000
2000
1000
900
800
700
600
400
200
0
aav9-ds-syn100-vh4-opti-hu-FKRP
AAV9-ds-syn100-FKRP-CpG(-)
AAV9-ds-CMP2.1-hu-FKRP
AAV9-ds-cmp2.1-hu-FKRP-CpG(-)
Uninjected control
Groups

FIG. 6 (con't)
C


Male 7.5months post injection (5 x 10^13 vg/kg)
Serum CK
10000
8000
6000
4000
2000
1000
900
800
700
600
400
200
0
aav9-ds-syn100-vh4-opti-hu-FKRP
AAV9-ds-syn100-FKRP-CpG(-)
AAV9-ds-CMP2.1-hu-FKRP
AAV9-ds-cmp2.1-hu-FKRP-CpG(-)
Uninjected control
Groups

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/011010

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/47(2006.01)i; **C12N 15/86**(2006.01)i; **A61K 38/00**(2006.01)i; **A61P 37/06**(2006.01)i; **A01K 67/027**(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 14/47(2006.01); A61K 31/047(2006.01); A61K 38/00(2006.01); A61K 48/00(2006.01); A61P 21/00(2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: human fukutin-related protein(FKRP), promoter, Vh4-Ig-intron3, poly A tail, optimization

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 10350305 B2 (THE CHARLOTTE-MECKLENBURG HOSPITAL AUTHORITY et al.) 16 July 2019 (2019-07-16) claim 1	14
A		1-8,15
A	WO 2019-008157 A1 (GENETHON et al.) 10 January 2019 (2019-01-10) the whole document	1-8,14,15
A	US 2019-0054037 A1 (THE CHARLOTTE MECKLENBURG HOSPITAL AUTHORITY D/B/A ATRIUM HEALTH) 21 February 2019 (2019-02-21) the whole document	1-8,14,15
A	XU, L. et al., 'Adeno-associated virus 9 mediated FKRP gene therapy restores functional glycosylation of α-dystroglycan and improves muscle functions', Molecular Therapy, 2013, Vol. 21, No. 10, pp. 1832-1840 the whole document	1-8,14,15

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 April 2022	**22 April 2022**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office **189 Cheongsa-ro, Seo-gu, Daejeon** **35208, Republic of Korea**	**HEO, Joo Hyung**
Facsimile No. **+82-42-481-8578**	Telephone No. **+82-42-481-5373**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GICQUEL, E. et al., 'AAV-mediated transfer of FKRP shows therapeutic efficacy in a murine model but requires control of gene expression', Human Molecular Genetics, 2017, Vol. 26, No. 10, pp. 1952-1965 the whole document	1-8,14,15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/011010

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/011010

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **28-34**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 28-34 pertain to a method for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

2. ☑ Claims Nos.: **10,12,13,17-20,23,30,34**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 10, 12, 13, 17-20, 23, 30 and 34 are regarded to be unclear because they refer to claim which does not comply with PCT Rule 6.4(a).

3. ☑ Claims Nos.: **9,11,16,21,22,24-29,31-33**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2022/011010

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
US 10350305 B2	16 July 2019	EP 3262065 A1	03 January 2018
		EP 3262065 A4	01 August 2018
		US 2017-0368199 A1	28 December 2017
		US 2019-0290781 A1	26 September 2019
		WO 2016-138387 A1	01 September 2016
WO 2019-008157 A1	10 January 2019	CA 3069045 A1	10 January 2019
		CN 110944656 A	31 March 2020
		EP 3648783 A1	13 May 2020
		JP 2020-530275 A	22 October 2020
		US 2021-0338837 A1	04 November 2021
US 2019-0054037 A1	21 February 2019	CA 3073194 A1	21 August 2020
		CA 3082651 A1	21 August 2020
		EP 3698624 A2	26 August 2020
		EP 3698624 A3	16 December 2020
		US 10245235 B2	02 April 2019
		US 10434072 B2	08 October 2019
		US 10434113 B2	08 October 2019
		US 10456367 B2	29 October 2019
		US 10561623 B2	18 February 2020
		US 10751298 B2	25 August 2020
		US 10993954 B2	04 May 2021
		US 2018-0169036 A1	21 June 2018
		US 2019-0008881 A1	10 January 2019
		US 2019-0070127 A1	07 March 2019
		US 2019-0091170 A1	28 March 2019
		US 2019-0380974 A1	19 December 2019
		US 2019-0381080 A1	19 December 2019
		US 2020-0179301 A1	11 June 2020
		US 2020-0267939 A1	27 August 2020
		US 2020-0267940 A1	27 August 2020