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(71) Applicants: **THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL** [US/US]; 109 Church Street, Chapel Hill, North Carolina 27516 (US). **THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA** [US/US]; 3160 Chestnut Street, Suite 200, Philadelphia, Pennsylvania 19104 (US).

(72) Inventors: **GRAY, Steven James**; 518 Timber Lake Drive, Southlake, Texas 76092 (US). **LYKKEN, Erik**; 1134 N Windomere Avenue, Dallas, Texas 75208 (US). **VITE, Charles H.**; 208 Rosenthal Building, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104 (US). **BRADBURY, Allison**; 846 N Ringgold Street, Philadelphia, Pennsylvania 19130 (US).

(74) Agent: **MYERS BIGEL, P.A.**; PO Box 37428, Raleigh, North Carolina 27627 (US).

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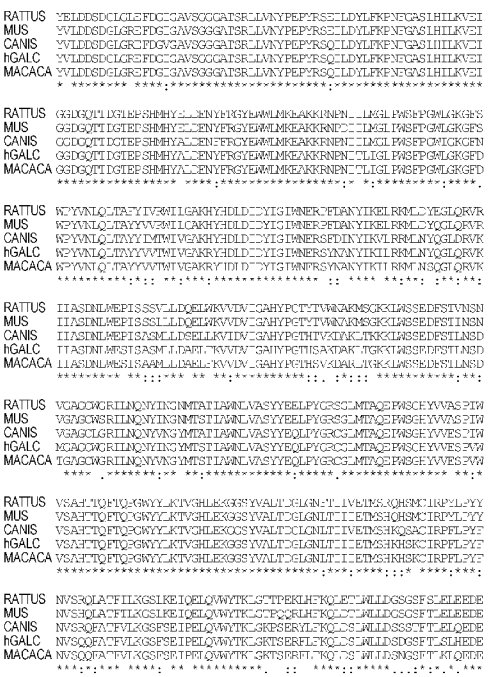
(54) Title: OPTIMIZED GALC GENES AND EXPRESSION CASSETTES AND THEIR USE

FIG. 1



(57) Abstract: This invention relates to polynucleotides comprising optimized GALC open reading frame (ORF) sequences, vectors comprising the same, and methods of using the same for delivery of the ORF to a cell or a subject and to treat disorders associated with aberrant expression of a GALC gene or aberrant activity of a GALC gene product in the subject, such as Krabbe disease (*i.e.*, globoid cell leukodystrophy (GLD)).

[Continued on next page]

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Optimized GALC Genes and Expression Cassettes and Their Use

STATEMENT OF PRIORITY

This application claims the benefit, under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 62/783,856, filed on December 21, 2018, the entire contents of which are incorporated by reference herein.

STATEMENT REGARDING ELECTRONIC FILING OF A SEQUENCE LISTING

A Sequence Listing in ASCII text format, submitted under 37 C.F.R. § 1.821, entitled 5470-861WO_ST25.txt, 56,729 bytes in size, generated on December 17, 2019 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.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant Number NS096087 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates to polynucleotides comprising optimized GALC open reading frame (ORF) sequences, vectors comprising the same, and methods of using the same for delivery of the ORF to a cell or a subject and to treat disorders associated with aberrant expression of a GALC gene or aberrant activity of a GALC gene product in the subject, such as Krabbe disease (KD) (*i.e.,* globoid cell leukodystrophy (GLD)).

BACKGROUND OF THE INVENTION

Krabbe disease (KD), also known as Globoid Cell Leukodystrophy (GLD), is a rapidly progressive, terminal lysosomal storage disorder caused by mutations in the *GALC* gene, primarily affecting the myelination of the nervous system. The *GALC* gene encodes a 669-amino acid, 17-exon, lysosomal enzyme galactosylceramidase (galactocerebrosidase) that catabolizes galactosylceramide (galactocerebroside), the principal glycosphingolipid of the myelin in brain tissue (Chen et al. 1993 Hum. Mol. Genet. 2(11):1841-1845). The GALC enzyme breaks down galactocerebroside to ceramide and galactose (Ferreira and Gahl 2017

Transl. Sci. Rare Dis. 2(12):1-71). GALC deficiency leads to accumulation of the substrate in the myelin sheath of the peripheral and central nervous system. Accumulated galactocerebroside in the white matter is catabolized via an alternative pathway, resulting in high-levels of psychosine that is toxic to oligodendrocytes and Schwann cells leading to apoptosis (reviewed by Ferreira and Gahl 2017; Bascou et al. 2018 Orphanet J. Rare Dis. 13(1):126). Galactocerebroside also accumulates in the cerebral microglial macrophages which fuse to form multinucleated globoid cells. Histopathology in the CNS is characterized by cerebral atrophy, loss of myelin, gliosis and globoid cells.

Classically, KD was categorized into four subgroups based on age at presentation: 1) Early Infantile (0-5 months), 2) Late-Infantile (6-36 months), 3) Juvenile (37 months – 16 years), and 4) Adult (>16 years). However, recent natural history data supports a revised classification for the Infantile sub-groups into 1) Infantile (</= 12 months) and 2) Late-Infantile (>12 months). Patients presenting within the first 12 months of life demonstrated a similar clinical severity and rate of neurodegeneration compared to those classically described as Early-Infantile (<6 months). All patients with onset of symptoms prior to 9 months and the majority of patients presenting between 9-12 months were consistent with the most severe KD-phenotype based on symptom onset, disease progression, and correlation with potential biomarkers (genotype, GALC enzyme activity, neurodevelopmental assessments, neuroimaging, and neurophysiological studies).

Psychosine is a substrate for GALC enzyme that accumulates in body fluids and tissues in the disease, and is used as a biomarker for KD. Newborn screening where dried blood spot (DBS) testing was done showed elevated psychosine levels in infants that developed infantile KD, but not in some asymptomatic patients (Chuang et al. 2013 Clin. Chim. Acta. 419:73-76; Turgeon et al. 2015 J. Inherit. Metab. Dis. 38(5):923-929). The elevated psychosine levels correlated with the enzyme activity in the lymphocytes and severity of the Krabbe disease (Liao et al. 2017 Clin. Chem. 63(8):1363-1369). All the newborns that had concentration of psychosine over 3 nmol/L in the DBS developed infantile KD (Escolar et al. 2017 Mol. Genet. Metab. 121(3):271-278). Clinical manifestations in KD exclusively impact the neuronal system. The more common and rapidly progressive form is an infantile onset associated with hypertonicity, evidenced by hyperactive reflexes early on which subsequently develop into hypotonic flaccidity, irritability, stiffness and seizures. Infantile and juvenile disease onset is associated with loss of acquired developmental milestones, motor deficits, and visual and hearing capabilities, while adult onset disease is

associated with abnormal gait, seizures and peripheral neuropathy. An abnormal increase in the T2 signal on magnetic resonance imaging (MRI) in the periventricular white matter, alterations in nerve conduction velocities and increased cerebrospinal fluid protein levels are seen following onset of symptoms. Regardless of subgroup, all clinical forms experience progressive central nervous system (CNS) dysfunction with significant psychomotor functional decline with progression of neurodegeneration and myelin destruction. Infantile-KD is the most clinically severe subgroup with presentation in the first year of life and rapid neurological decline. Most Infantile-KD patients present with axial hypotonia, irritability, and loss of acquired developmental milestones. Further neurologic deterioration is rapid after the onset of symptoms in this group. Most Infantile KD patients succumb to the disease by 2 years of age.

Treatment options for KD are currently limited to hematopoietic stem cell therapy (HSCT) (Escolar et al. 2005 N. Engl. J. Med. 352(20):2069-2081; Lim et al. 2008 Bone Marrow Transplant. 41(9):831-832; Escolar et al. 2006 CML: Lysosomal Storage Disease 6(3):72-79; Escolar et al. 2006 Pediatrics 118(3):e879-889; Krivit et al. 1998 N. Engl. J. Med. 338(16):1119-1126). While HSCT is associated with some functional improvements particularly in mobility, communication and feeding (Escolar et al. 2005 N. Engl. J. Med.; Allewelt et al. 2018 Biol. Blood Marrow Transplant. S1083-8791(18)30334-3; Shapiro et al. 1995 J. Inherit. Metab. Dis. 18(4):413-429; Kwon et al. 2018 Orphanet J. Rare Dis. 13(1):30), this treatment has substantial limitations. HSCT has only shown to be effective if performed within 30 days of birth prior to the onset of disease (Allewelt et al. 2018; Kwon et al. 2018). The timing of stem cell engraftment is approximately 2 months, which is not ideal in a rapidly progressive degenerative disease. HSCT procedure itself in pediatric populations is associated with a 20% risk of mortality (Shin et al. 2016 J. Neurosci. 36(6):1858-1870; Bonkowsky et al. 2018 J. Child Neurol. 33(14):882-887). Graft-versus-host disease (GVHD), another complication of HSCT, impacted about 10% of the pediatric transplant recipients with inherited metabolic disorders (Prasad et al. 2008 Blood. 112(7):2979-2989). Further, HSCT requires a prolonged hospitalization that contributes to poorer neurodevelopmental outcomes (Syed et al. 2016 Hematol. Oncol. Clin. North Am. 30(4):887-898). Based on a meta-analysis of HSCT for leukodystrophies, complications, cause of death, and poor outcomes appear to be under-reported in the published literature (Musolino et al. 2014 Neuropediatrics 45(3):169-174). Further, it is not clear whether poor outcomes are related to disease progression or are inherent to the required myeloablative procedures, which are

known to cause long-term neurologic dysfunction (Syed et al. 2016). Pre-symptomatic intervention with HSCT in KD results in increased survival, but quality of life remains poor with a progressive peripheral neuropathy that is ultimately fatal (Aldenhoven and Kurtzberg 2015 Cytotherapy 17(6):765-774). In a 15-year follow-up study of 18 Infantile KD patients treated with HSCT within the first 7 weeks of life, 5 died (3 peri-treatment, 1 due to disease progression, 1 unrelated), 17 required special education services, 2 required an augmentative assistive communication device, all had some degree of spasticity, and 7 required some type of assistive device for ambulation/mobility (Wright et al. 2017 Neurology 89(13):1365-1372). The New York State newborn screening identified five infants at high risk for infantile KD; one died without HSCT, two died of complications from the procedure, and the two alive have significant GVHD (Wenger et al. 2016 J. Neurosci. Res. 94(11):982-989).

There remains a need in the art for an effective treatment that targets the cause of the disease, *i.e., GALC* gene mutations. The present invention overcomes shortcomings in the art by providing codon-optimized GALC genes, expression cassettes, and vectors capable of providing therapeutic levels of GALC expression for treating disorders associated with GALC expression such as KD.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the development of optimized GALC genes, expression cassettes, and vectors capable of providing therapeutic levels of GALC expression for treating disorders associated with GALC expression such as Krabbe disease.

Thus, one aspect of the invention relates to a polynucleotide comprising a canine or human GALC open reading frame, wherein the canine or human GALC open reading frame has been codon-optimized for expression in canine or human cells.

A further aspect of the invention relates to an expression cassette comprising a polynucleotide comprising a canine or human GALC open reading frame and vectors, transformed cells, and transgenic animals comprising the polynucleotide of the invention.

Another aspect of the invention relates to a pharmaceutical formulation comprising the polynucleotide, expression cassette, vector, and/or transformed cell of the invention in a pharmaceutically acceptable carrier.

An additional aspect of the invention relates to a method of expressing a GALC open reading frame in a cell, comprising contacting the cell with the polynucleotide, expression

cassette, and/or vector of the invention, thereby expressing the GALC open reading frame in the cell.

A further aspect of the invention relates to a method of expressing a GALC open reading frame in a subject, comprising delivering to the subject the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, thereby expressing the GALC open reading frame in the subject.

An additional aspect of the invention relates to a method of treating a disorder associated with aberrant expression of an GALC gene or aberrant activity of an GALC gene product in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, such that the GALC open reading frame is expressed in the subject.

A further aspect of the invention relates to a method of treating Krabbe disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, such that the GALC open reading frame is expressed in the subject.

Another aspect of the invention relates to a polynucleotide, expression cassette, vector, and/or transformed cell of the invention for use in a method of treating a disorder associated with aberrant expression of a GALC gene or aberrant activity of a GALC gene product in a subject in need 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 shows similarities in GALC protein sequence between different species. Human (homo; optimized; SEQ ID NO:15) GALC protein mature sequence (no signal peptide) compared to the mouse (mus; SEQ ID NO:13; 83.49%), rat (rattus; SEQ ID NO:12; 82.55%), dog (canis; SEQ ID NO:14; 89.42%) and monkey (macaca; SEQ ID NO:16; 97.20%) retain high level of amino acid identity. The asterisk (*) annotates a fully conserved amino acid residue, colon (:) annotates strongly similar residues and period (.) annotates weakly similar residues. Amino acids that are not conserved are not annotated.

FIG. 2 shows that AAV9/mGALC prevents demyelination. Top panel: Sciatic nerves from PND35 age-matched mice treated at PND11 with vehicle or AAV9/mGALC. Sections stained with toluidine blue detects the preserved myelin around the axons in treated mice

(ssAAV9) compared to loss in myelin around degenerating axons in untreated (vehicle). Bottom panel: Luxol fast blue staining of myelin in Cerebellum, cervical and lumbar regions of the CNS in untreated (vehicle) compared to treated mice (ssAAV9) demonstrates well-preserved myelin in mice that received the gene therapy.

FIGS. 3A-3C show that nerve conduction velocity (NCV) is retained in AAV9/cGALC treated Krabbe dogs. Dogs received a single IT-CM AAV9/cGALC per the group assignment, untreated dogs were not dosed. NCV testing was repeated every 8 weeks following dose administration. Conduction was evaluated in pelvic limb (**FIG. 3A**), thoracic limb (**FIG. 3B**) and sensory nerve (**FIG. 3C**). Untreated (red; n = 2) or those administered high-dose at 2 weeks age (green; n = 4) are plotted on the graph. Average NCV in wild type (Normal) and untreated dogs is indicated by black lines in the figure. Note: Untreated dogs only had one session as they reached humane endpoint and did not survive until the next session.

FIG. 4 shows AAV9/cGALC reduces CSF protein concentration in Krabbe dogs.

FIG 5 shows that gene therapy preserves cerebral white matter. Brain MRIs are representative T2-weighed scans from one animal from each cohort. The brain of a Krabbe affected dogs shows T2-weighted bilaterally symmetrical increases in signal intensity of the corona radiata (**FIG. 5 panel D, arrow**), corpus callosum, centrum semiovale, internal capsule (**FIG. 5 panel E, arrow**), and cerebellar white matter (**FIG. 5 panel F, arrow**) when compared to a normal, age-matched control dogs (**FIG. 5 panels A-C).** Cerebral ventricles are dilated (**FIG. 5 panel E, arrow**) and sulci are widened (**FIG. 5 panel D, arrow**) indicating cerebral atrophy in Krabbe dogs. In contrast, 16 weeks after IT-CM delivery of AAV9-cGALC white matter signal remains normal at the corpus callosum and internal capsule, although isointensity is noted at the corona radiata and centrum semiovale. Cerebellar white matter is preserved. Ventricles and sulci remain within normal limits indicating attenuation of brain atrophy. Notably, stabilization of MRI changes is seen at 52 weeks of age (**FIG. 5 panels J-L**).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is explained in greater detail below. 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. 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 specification is intended to illustrate some particular embodiments of the invention, and not to exhaustively specify all permutations, combinations and variations thereof.

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 complex 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.

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.

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).

All publications, patent applications, patents, nucleotide sequences, amino acid sequences and other references mentioned herein are incorporated by reference in their entirety.

Definitions

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.

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").

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.

Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound or agent of this invention, dose, time, temperature, and the like, is meant to encompass variations of ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount.

As used herein, the transitional phrase "consisting essentially of" is to be interpreted as encompassing the recited materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising."

The term "consists essentially of" (and grammatical variants), as applied to a polynucleotide or polypeptide sequence of this invention, means a polynucleotide or polypeptide that consists of both the recited sequence (*e.g.,* SEQ ID NO) and a total of ten or less (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional nucleotides or amino acids on the 5' and/or 3' or N-terminal and/or C-terminal ends of the recited sequence or between the two ends (*e.g.,* between domains) such that the function of the polynucleotide or polypeptide is not materially altered. The total of ten or less additional nucleotides or amino acids includes the total number of additional nucleotides or amino acids added together. The term "materially altered," as applied to polynucleotides of the invention, refers to an increase or decrease in ability to express the encoded polypeptide of at least about 50% or more as compared to the expression level of a polynucleotide consisting of the recited sequence. The term "materially altered," as applied to polypeptides of the invention, refers to an increase or decrease in

biological activity of at least about 50% or more as compared to the activity of a polypeptide consisting of the recited sequence.

The term "parvovirus" as used herein encompasses the family *Parvoviridae*, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus*, *Erythrovirus*, *Densovirus*, *Iteravirus*, and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, minute virus of mouse, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, H1 parvovirus, muscovy duck parvovirus, snake parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art. *See, e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers).

The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, 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, AAV type 12, AAV type 13, avian AAV, bovine AAV, canine AAV, goat AAV, snake AAV, equine AAV, and ovine AAV. *See, e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapter 69 (4th ed., Lippincott-Raven Publishers); and **Table 1**.

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 and **Table 1**), which are also encompassed by the term "AAV."

The parvovirus particles and genomes of the present invention can be from, but are not limited to, AAV. The genomic sequences of various serotypes of AAV and the autonomous parvoviruses, as well as the sequences of the native 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 GenBank. *See, e.g.,* GenBank Accession Numbers NC_002077, NC_001401, NC_001729, NC_001863, NC_001829, NC_001862, NC_000883, NC_001701, NC_001510, NC_006152, NC_006261, AF063497, U89790, AF043303, AF028705, AF028704, J02275, J01901, J02275, X01457, AF288061, AH009962, AY028226,

AY028223, AY631966, AX753250, EU285562, NC_001358, NC_001540, AF513851, AF513852 and AY530579; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also, e.g.,* Bantel-Schaal *et al.*, (1999) *J. Virol.* 73: 939; Chiorini *et al.*, (1997) *J. Virol.* 71:6823; Chiorini *et al.*, (1999) *J. Virol.* 73:1309; Gao *et al.*, (2002) *Proc. Nat. Acad. Sci. USA* 99:11854; Moris *et al.*, (2004) *Virol.* 33-:375-383; Mori *et al.*, (2004) *Virol.* 330:375; Muramatsu *et al.*, (1996) *Virol.* 221:208; Ruffing *et al.*, (1994) *J. Gen. Virol.* 75:3385; Rutledge *et al.*, (1998) *J. Virol.* 72:309; Schmidt *et al.*, (2008) *J. Virol.* 82:8911; Shade *et al.*, (1986) *J. Virol.* 58:921; Srivastava *et al.*, (1983) *J. Virol.* 45:555; Xiao *et al.*, (1999) *J. Virol.* 73:3994; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Patent No. 6,156,303; the disclosures of which are incorporated by reference herein for teaching parvovirus and AAV nucleic acid and amino acid sequences. *See also* **Table 1**. An early description of the AAV1, AAV2 and AAV3 ITR 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 "chimeric" AAV nucleic acid capsid coding sequence or AAV capsid protein is one that combines portions of two or more capsid sequences. A "chimeric" AAV virion or particle comprises a chimeric AAV capsid protein.

The term "tropism" as used herein refers to preferential entry of the virus into certain cell or tissue type(s) and/or preferential interaction with the cell surface that facilitates entry into certain cell or tissue types, optionally and preferably followed by expression (*e.g.,* transcription and, optionally, translation) of sequences carried by the viral genome in the cell, *e.g.,* for a recombinant virus, expression of the heterologous nucleotide sequence(s). Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, *e.g.,* for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of a rAAV genome, gene expression from the viral genome may be from a stably integrated provirus and/or from a non-integrated episome, as well as any other form which the virus nucleic acid may take within the cell.

The term "tropism profile" refers to the pattern of transduction of one or more target cells, tissues and/or organs. Representative examples of chimeric AAV capsids have a tropism profile characterized by efficient transduction of cells of the central nervous system

(CNS) with only low transduction of peripheral organs (*see e.g.,* US Patent No. 9,636,370 McCown *et al.*, and US patent publication 2017/0360960 Gray *et al.*).

The term "disorder associated with aberrant expression of a GALC gene" as used herein refers to a disease, disorder, syndrome, or condition that is caused by or a symptom of decreased or altered expression of the GALC gene in a subject relative to the expression level in a normal subject or in a population.

The term "disorder associated with aberrant activity of a GALC gene product" as used herein refers to a disease, disorder, syndrome, or condition that is caused by or a symptom of decreased or altered activity of the GALC gene product in a subject relative to the activity in a normal subject or in a population.

As used herein, "transduction" of a cell by a virus vector (*e.g.,* an AAV vector) means entry of the vector into the cell and transfer of genetic material into the cell by the incorporation of nucleic acid into the virus vector and subsequent transfer into the cell via the virus vector.

Unless indicated otherwise, "efficient transduction" or "efficient tropism," or similar terms, can be determined by reference to a suitable positive or negative control (*e.g.,* at least about 50%, 60%, 70%, 80%, 85%, 90%, 95% or more of the transduction or tropism, respectively, of a positive control or at least about 110%, 120%, 150%, 200%, 300%, 500%, 1000% or more of the transduction or tropism, respectively, of a negative control).

Similarly, it can be determined if a virus "does not efficiently transduce" or "does not have efficient tropism" for a target tissue, or similar terms, by reference to a suitable control. In particular embodiments, the virus vector does not efficiently transduce (*i.e.,* does not have efficient tropism for) tissues outside the CNS, *e.g.,* liver, kidney, gonads and/or germ cells. In particular embodiments, undesirable transduction of tissue(s) (*e.g.,* liver) is 20% or less, 10% or less, 5% or less, 1% or less, 0.1% or less of the level of transduction of the desired target tissue(s) (*e.g.,* CNS cells).

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.

A "polynucleotide," "nucleic acid," or "nucleotide sequence" may be of RNA, DNA or DNA-RNA hybrid sequences (including both naturally occurring and non-naturally occurring nucleotides), but is preferably either a single or double stranded DNA sequence.

The term "regulatory element" refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, etc. The region in a nucleic acid sequence or polynucleotide in which one or more regulatory elements are found may be referred to as a "regulatory region."

As used herein with respect to nucleic acids, the term "operably linked" refers to a functional linkage between two or more nucleic acids. For example, a promoter sequence may be described as being "operably linked" to a heterologous nucleic acid sequence because the promoter sequences initiates and/or mediates transcription of the heterologous nucleic acid sequence. In some embodiments, the operably linked nucleic acid sequences are contiguous and/or are in the same reading frame.

The term "open reading frame (ORF)," as used herein, refers to the portion of a polynucleotide, *e.g.,* a gene, that encodes a polypeptide. The term "coding region" may be used interchangeably with open reading frame.

The term "codon-optimized," as used herein, refers to a gene coding sequence that has been optimized to increase expression by substituting one or more codons normally present in a coding sequence (for example, in a wild-type sequence, including, *e.g.,* a coding sequence for GALC) with a codon for the same (synonymous) amino acid. In this manner, the protein encoded by the gene is identical, but the underlying nucleobase sequence of the gene or corresponding mRNA is different. In some embodiments, the optimization substitutes one or more rare codons (that is, codons for tRNA that occur relatively infrequently in cells from a particular species) with synonymous codons that occur more frequently to improve the efficiency of translation. For example, in human codon-optimization one or more codons in a coding sequence are replaced by codons that occur more frequently in human cells for the same amino acid. Codon optimization can also increase gene expression through other

mechanisms that can improve efficiency of transcription and/or translation. Strategies include, without limitation, increasing total GC content (that is, the percent of guanines and cytosines in the entire coding sequence), decreasing CpG content (that is, the number of CG or GC dinucleotides in the coding sequence), removing cryptic splice donor or acceptor sites, and/or adding or removing ribosomal entry sites, such as Kozak sequences. Desirably, a codon-optimized gene exhibits improved protein expression, for example, the protein encoded thereby is expressed at a detectably greater level in a cell compared with the level of expression of the protein provided by the wild-type gene in an otherwise similar cell.

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.

Table 1:

AAV Serotypes/Isolates	GenBank Accession Number		AAV Serotypes/Isolates	GenBank Accession Number		AAV Serotypes/Isolates	GenBank Accession Number
Clonal Isolates			Hu S17	AY695376		Cy3	AY243019
Avian AAV ATCC VR-865	AY186198, AY629583, NC_004828		Hu T88	AY695375		Cy5	AY243017
Avian AAV strain DA-1	NC_006263, AY629583		Hu T71	AY695374		Rh13	AY243013
Bovine AAV	NC_005889, AY388617		Hu T70	AY695373			
AAV4	NC_001829		Hu T40	AY695372		**Clade E**	
AAV5	AY18065, AF085716		Hu T32	AY695371		Rh38	AY530558
Rh34	AY243001		Hu T17	AY695370		Hu66	AY530626
Rh33	AY243002		Hu LG15	AY695377		Hu42	AY530605
Rh32	AY243003					Hu67	AY530627
AAV10	AY631965		**Clade C**			Hu40	AY530603
AAV11	AY631966		**AAV 3**	NC_001729		Hu41	AY530604
AAV12	DQ813647		**AAV 3B**	NC_001863		Hu37	AY530600
AAV13	EU285562		Hu9	AY530629		Rh40	AY530559
			Hu10	AY530576		Rh2	AY243007
Clade A			Hu11	AY530577		Bb1	AY243023
AAV1	NC_002077, AF063497		Hu53	AY530615		Bb2	AY243022
AAV6	NC_001862		Hu55	AY530617		**Rh10**	AY243015
Hu.48	AY530611		Hu54	AY530616		Hu17	AY530582
Hu 43	AY530606		Hu7	AY530628		Hu6	AY530621
Hu 44	AY530607		Hu18	AY530583		Rh25	AY530557
Hu 46	AY530609		Hu15	AY530580		Pi2	AY530554
			Hu16	AY530581		Pi1	AY530553
Clade B			Hu25	AY530591		Pi3	AY530555
Hu19	AY530584		Hu60	AY530622		Rh57	AY530569
Hu20	AY530586		Ch5	AY243021		Rh50	AY530563
Hu23	AY530589		Hu3	AY530595		Rh49	AY530562
Hu22	AY530588		Hu1	AY530575		Hu39	AY530601
Hu24	AY530590		Hu4	AY530602		Rh58	AY530570
Hu21	AY530587		Hu2	AY530585		Rh61	AY530572
Hu27	AY530592		Hu61	AY530623		Rh52	AY530565
Hu28	AY530593					Rh53	AY530566
Hu29	AY530594		**Clade D**			Rh51	AY530564
Hu63	AY530624		Rh62	AY530573		Rh64	AY530574
Hu64	AY530625		Rh48	AY530561		Rh43	AY530560
Hu13	AY530578		Rh54	AY530567		**AAV8**	AF513852
Hu56	AY530618		Rh55	AY530568		Rh8	AY242997
Hu57	AY530619		Cy2	AY243020		Rh1	AY530556
Hu49	AY530612		**AAV7**	AF513851			
Hu58	AY530620		Rh35	AY243000		**Clade F**	
Hu34	AY530598		Rh37	AY242998		**AAV9** (Hu14)	AY530579
Hu35	AY530599		Rh36	AY242999		Hu31	AY530596
AAV2	NC_001401		Cy6	AY243016		Hu32	AY530597
Hu45	AY530608		Cy4	AY243018			
Hu47	AY530610						
Hu51	AY530613						
Hu52	AY530614						
Hu T41	AY695378						

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 one embodiment, 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.

As used herein, an "isolated" nucleic acid or nucleotide sequence (*e.g.,* an "isolated DNA" or an "isolated RNA") means a nucleic acid or nucleotide sequence separated or substantially free from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the nucleic acid or nucleotide sequence.

Likewise, an "isolated" polypeptide means a polypeptide that is separated or substantially free from at least some of the other components of the naturally occurring

organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

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.

As used herein, by "isolate" (or grammatical equivalents) a virus vector, it is meant that the virus vector is at least partially separated from at least some of the other components in the starting material.

By the term "treat," "treating," or "treatment of" (or grammatically equivalent terms) is meant to reduce or to at least partially improve or ameliorate the severity of the subject's condition and/or to alleviate, mitigate or decrease in at least one clinical symptom and/or to delay the progression of the condition.

As used herein, the term "prevent," "prevents," or "prevention" (and grammatical equivalents thereof) means to delay or inhibit the onset of a disease. The terms are not meant to require complete abolition of disease, and encompass any type of prophylactic treatment to reduce the incidence of the condition or delays the onset of the condition.

A "treatment 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.

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.

A "heterologous nucleotide sequence" or "heterologous nucleic acid," with respect to a virus, is a sequence or nucleic acid, respectively, that is not naturally occurring in the virus. Generally, the heterologous nucleic acid or nucleotide sequence comprises an open reading frame that encodes a polypeptide and/or a nontranslated RNA.

A "vector" refers to a compound used as a vehicle to carry foreign genetic material into another cell, where it can be replicated and/or expressed. A cloning vector containing foreign nucleic acid is termed a recombinant vector. Examples of nucleic acid vectors are plasmids, viral vectors, cosmids, expression cassettes, and artificial chromosomes. Recombinant vectors typically contain an origin of replication, a multicloning site, and a selectable marker. The nucleic acid sequence typically consists of an insert (recombinant nucleic acid or transgene) and a larger sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Expression vectors (expression constructs or expression cassettes) are for the expression of the exogenous gene in the target cell, and generally have a promoter sequence that drives expression of the exogenous gene/ORF. Insertion of a vector into the target cell is referred to transformation or transfection for bacterial and eukaryotic cells, although insertion of a viral vector is often called transduction. The term "vector" may also be used in general to describe items to that serve to carry foreign genetic material into another cell, such as, but not limited to, a transformed cell or a nanoparticle.

As used herein, the term "vector," "virus vector," "delivery vector" (and similar terms) in a specific embodiment 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 as described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Thus, in some embodiments, double stranded (duplex) genomes can be packaged.

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 (ITR) (*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.*

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).

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 (*see, e.g.,* **Table 1**). 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 virus vectors of the invention can further be "targeted" virus vectors (*e.g.,* having a directed tropism) and/or a "hybrid" parvovirus (*i.e.,* in which the viral ITRs and viral capsid

are from different parvoviruses) as described in international patent publication WO 00/28004 and Chao *et al.*, (2000) *Mol. Therapy* 2:619.

Further, the viral capsid or genomic elements can contain other modifications, including insertions, deletions and/or substitutions.

As used herein, the term "amino acid" encompasses any naturally occurring amino acids, modified forms thereof, and synthetic amino acids, including non-naturally occurring amino acids.

Naturally occurring, levorotatory (L-) amino acids are shown in **Table 2**.

Table 2

Amino Acid Residue	Abbreviation	
	Three-Letter Code	One-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid (Aspartate)	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid (Glutamate)	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Alternatively, the amino acid can be a modified amino acid residue (nonlimiting examples are shown in **Table 3**) or can be an amino acid that is modified by post-translation modification (*e.g.,* acetylation, amidation, formylation, hydroxylation, methylation, phosphorylation or sulfatation).

Further, the non-naturally occurring amino acid can be an "unnatural" amino acid as described by Wang *et al.*, (2006) *Annu. Rev. Biophys. Biomol. Struct.* 35:225-49. These unnatural amino acids can advantageously be used to chemically link molecules of interest to the AAV capsid protein.

Table 3: Amino Acid Residue Derivatives

Modified Amino Acid Residue	Abbreviation
2-Aminoadipic acid	Aad
3-Aminoadipic acid	bAad
beta-Alanine, beta-Aminoproprionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, Piperidinic acid	4Abu
6-Aminocaproic acid	Acp
2-Aminoheptanoic acid	Ahe
2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	bAib
2-Aminopimelic acid	Apm
t-butylalanine	t-BuA
Citrulline	Cit
Cyclohexylalanine	Cha
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,2'-Diaminopimelic acid	Dpm
2,3-Diaminoproprionic acid	Dpr
N-Ethylglycine	EtGly
N-Ethylasparagine	EtAsn
Homoarginine	hArg
Homocysteine	hCys
Homoserine	hSer
Hydroxylysine	Hyl
Allo-Hydroxylysine	aHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	aIle
Methionine sulfoxide	MSO
N-Methylglycine, sarcosine	MeGly
N-Methylisoleucine	MeIle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal
2-Naphthylalanine	2-Nal
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn
4-Chlorophenylalanine	Phe(4-Cl)
2-Fluorophenylalanine	Phe(2-F)
3-Fluorophenylalanine	Phe(3-F)
4-Fluorophenylalanine	Phe(4-F)
Phenylglycine	Phg
Beta-2-thienylalanine	Thi

The term "template" or "substrate" is used herein to refer to a polynucleotide sequence that may be replicated to produce the parvovirus viral DNA. For the purpose of vector production, the template will typically be embedded within a larger nucleotide sequence or construct, including but not limited to a plasmid, naked DNA vector, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC) or a viral vector (*e.g.*, adenovirus,

herpesvirus, Epstein-Barr Virus, AAV, baculoviral, retroviral vectors, and the like). Alternatively, the template may be stably incorporated into the chromosome of a packaging cell.

As used herein, parvovirus or AAV "Rep coding sequences" indicate the nucleic acid sequences that encode the parvoviral or AAV non-structural proteins that mediate viral replication and the production of new virus particles. The parvovirus and AAV replication genes and proteins have been described in, *e.g.,* FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

The "Rep coding sequences" need not encode all of the parvoviral or AAV Rep proteins. For example, with respect to AAV, the Rep coding sequences do not need to encode all four AAV Rep proteins (Rep78, Rep 68, Rep52 and Rep40), in fact, it is believed that AAV5 only expresses the spliced Rep68 and Rep40 proteins. In representative embodiments, the Rep coding sequences encode at least those replication proteins that are necessary for viral genome replication and packaging into new virions. The Rep coding sequences will generally encode at least one large Rep protein (*i.e.,* Rep78/68) and one small Rep protein (*i.e.,* Rep52/40). In particular embodiments, the Rep coding sequences encode the AAV Rep78 protein and the AAV Rep52 and/or Rep40 proteins. In other embodiments, the Rep coding sequences encode the Rep68 and the Rep52 and/or Rep40 proteins. In a still further embodiment, the Rep coding sequences encode the Rep68 and Rep52 proteins, Rep68 and Rep40 proteins, Rep78 and Rep52 proteins, or Rep78 and Rep40 proteins.

As used herein, the term "large Rep protein" refers to Rep68 and/or Rep78. Large Rep proteins of the claimed invention may be either wild-type or synthetic. A wild-type large Rep protein may be from any parvovirus or AAV, including but not limited to serotypes 1, 2, 3a, 3b, 4, 5, 6, 7, 8, 9, 10, 11, or 13, or any other AAV now known or later discovered (*see, e.g.,* **Table 1**). A synthetic large Rep protein may be altered by insertion, deletion, truncation and/or missense mutations.

Those skilled in the art will further appreciate that it is not necessary that the replication proteins be encoded by the same polynucleotide. For example, for MVM, the NS-1 and NS-2 proteins (which are splice variants) may be expressed independently of one another. Likewise, for AAV, the p19 promoter may be inactivated and the large Rep protein(s) expressed from one polynucleotide and the small Rep protein(s) expressed from a different polynucleotide. Typically, however, it will be more convenient to express the replication proteins from a single construct. In some systems, the viral promoters (*e.g.,* AAV

p19 promoter) may not be recognized by the cell, and it is therefore necessary to express the large and small Rep proteins from separate expression cassettes. In other instances, it may be desirable to express the large Rep and small Rep proteins separately, *i.e.,* under the control of separate transcriptional and/or translational control elements. For example, it may be desirable to control expression of the large Rep proteins, so as to decrease the ratio of large to small Rep proteins. In the case of insect cells, it may be advantageous to down-regulate expression of the large Rep proteins (*e.g.,* Rep78/68) to avoid toxicity to the cells (*see, e.g.,* Urabe *et al.*, (2002) *Human Gene Therapy* 13:1935).

As used herein, the parvovirus or AAV "cap coding sequences" encode the structural proteins that form a functional parvovirus or AAV capsid (*i.e.,* can package DNA and infect target cells). Typically, the cap coding sequences will encode all of the parvovirus or AAV capsid subunits, but less than all of the capsid subunits may be encoded as long as a functional capsid is produced. Typically, but not necessarily, the cap coding sequences will be present on a single nucleic acid molecule.

The capsid structure of autonomous parvoviruses and AAV are described in more detail in BERNARD N. FIELDS *et al.*, VIROLOGY, volume 2, chapters 69 & 70 (4th ed., Lippincott-Raven Publishers).

By "substantially retain" a property, it is meant that at least about 75%, 85%, 90%, 95%, 97%, 98%, 99% or 100% of the property (*e.g.,* activity or other measurable characteristic) is retained.

GALC Expression Cassettes and Vectors

The present invention relates to the design of a GALC expression cassette to provide therapeutic levels of expression of galactocerebrosidase, the lysosomal enzyme encoded by the GALC gene, and the use of the expression cassette to achieve therapeutic levels of GALC in a subject.

Thus, one aspect of the invention relates to a polynucleotide comprising a mammalian GALC open reading frame (ORF), wherein the GALC open reading frame has been codon-optimized for expression in mammalian cells. The term "mammal" as used herein includes, but is not limited to, humans, primates, non-human primates (*e.g.,* monkeys and baboons), cattle, sheep, goats, pigs, horses, cats, dogs, rabbits, rodents (*e.g.,* rats, mice, hamsters, and the like), *etc.* The open reading frame is the portion of the GALC gene that encodes GALC. In some embodiments, the mammalian GALC open reading frame may be a human or a

canine CALC open reading frame. As used herein, a mammalian GALC ORF refers to a nucleotide sequence that encodes mammalian GALC, *e.g.,* a human or a canine CALC ORF refers to a nucleotide sequence that encodes a human or a canine GALC. Codon optimization is a technique well known in the art and optimal codons for expression in different species are known. The use of a codon-optimized GALC sequence allows one to distinguish expression of the transduced sequence from expression of the endogenous GALC sequence in a subject.

In some embodiments, the codon-optimized GALC open reading frame encodes a GALC enzyme that is modified from the wild-type sequence, *e.g.,* comprises, consists essentially of, or consists of an amino acid sequence in which 1, 2, 3, 4, or 5 residues have been substituted, added, and/or deleted compared to the wild-type amino acid sequence.

In some embodiments, the codon-optimized GALC open reading frame comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:1 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:1. Human codon-optimized GALC open reading frame

ATGGCTGAGTGGCTCCTTAGCGCGAGCTGGCAGCGGAGAGCCAAGGCAATGACA
GCGGCGGCGGGCTCCGCCGGACGCGCTGCCGTCCCTCTGTTGCTCTGTGCGTTGC
TGGCACCGGGTGGAGCGTATGTGCTTGATGATTCGGACGGACTCGGTAGAGAAT
TTGACGGAATCGGAGCGGTCAGCGGTGGAGGAGCGACGAGCCGCCTGCTCGTGA
ACTATCCCGAACCCTACCGATCCCAGATTCTGGACTACCTTTTCAAACCTAACTT
CGGCGCAAGCCTTCACATCCTCAAGGTGGAGATCGGTGGGGACGGTCAGACCAC
AGACGGTACGGAACCATCGCACATGCACTATGCGCTCGACGAAAACTACTTTAG
AGGGTATGAGTGGTGGCTGATGAAAGAGGCCAAAAAGCGGAATCCGAATATCAC
TCTCATTGGTTTGCCGTGGAGCTTCCCCGGCTGGCTGGGGAAGGGGTTCGACTGG
CCCTATGTGAACCTTCAACTGACAGCGTATTACGTGGTCACATGGATTGTCGGGG
CGAAGAGGTATCATGACTTGGATATCGACTATATTGGTATCTGGAACGAGAGATC
CTACAACGCAAACTACATCAAAATCCTTAGAAAGATGTTGAATTATCAGGGGCT
GCAGAGAGTCAAAATCATCGCATCCGACAATCTTTGGGAATCGATCTCAGCGTCA
ATGCTCCTCGACGCGGAACTGTTTAAAGTGGTGGATGTCATTGGGGCGCATTACC
CGGGAACACACTCGGCGAAAGACGCAAAGTTGACGGGGAAGAAATTGTGGTCG
AGCGAGGATTTTTCCACTCTTAATTCGGATATGGGGGCAGGGTGTTGGGGAAGA
ATTCTGAACCAGAACTATATCAACGGGTATATGACCTCGACGATCGCCTGGAATC

TTGTGGCATCCTACTACGAGCAGCTGCCTTACGGGAGGTGCGGTCTTATGACAGC
GCAGGAGCCCTGGTCGGGACATTACGTCGTCGAGAGCCCCGTATGGGTATCAGC
CCACACGACCCAGTTTACACAGCCGGGCTGGTATTACCTTAAGACGGTGGGCCAT
CTTGAGAAGGGAGGTAGCTATGTCGCGCTGACGGATGGCTTGGGTAATTTGACA
ATCATCATTGAAACTATGTCGCATAAACACTCAAAGTGCATTCGCCCTTTTCTGC
CCTATTTCAACGTCAGCCAGCAATTTGCGACGTTTGTGCTTAAGGGATCGTTTTCG
GAGATTCCCGAACTTCAGGTCTGGTACACGAAACTTGGAAAGACGTCAGAAAGG
TTCCTTTTCAAGCAGTTGGACTCGCTCTGGCTTTTGGATAGCGACGGATCGTTCAC
TCTGTCCTTGCACGAGGATGAGTTGTTCACGCTCACTACCCTCACCACTGGCAGA
AAGGGCTCCTACCCGTTGCCCCCGAAAAGCCAGCCGTTTCCTTCAACTTATAAGG
ATGACTTTAATGTCGATTACCCATTCTTCTCGGAGGCCCCGAATTTTGCCGACCA
AACAGGAGTATTTGAATACTTCACGAACATCGAGGACCCGGGGGAGCACCATTT
CACTCTGAGACAAGTGTTGAACCAAAGGCCGATTACTTGGGCAGCCGATGCCAG
CAATACCATTTCGATTATCGGAGACTATAACTGGACAAACTTGACCATCAAATGC
GATGTCTATATCGAAACGCCTGATACAGGGGGTGTGTTCATCGCTGGTCGCGTAA
ACAAAGGGGGAATTTTGATCCGCTCAGCTAGAGGGATCTTCTTTTGGATTTTCGC
GAACGGAAGCTACCGCGTGACGGGAGACTTGGCGGGATGGATCATCTACGCCCT
GGGTCGCGTGGAGGTAACAGCGAAAAAGTGGTACACGTTGACCTTGACAATTAA
GGGGCACTTCACGTCCGGGATGCTGAACGACAAGAGCCTCTGGACGGACATCCC
CGTGAATTTCCCCAAAAACGGGTGGGCAGCAATTGGGACGCACTCCTTTGAATTT
GCGCAATTCGACAACTTTTTGGTAGAGGCTACGCGG

In some embodiments, the codon-optimized GALC open reading frame comprises, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:2 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:2. Canine codon-optimized GALC open reading frame

ATGACCGCAGCCGCAGGATCTGCAGGCCATGCTGCGGTGCCCCTGTTGTTGTGTG
CCCTTCTGGTCCCTGGCGGAGCTTACGTGCTGGACGACTCCGACGGTTTGGGCCG
GGAGTTCGACGGAGTGGGAGCTGTCTCCGGTGGTGGAGCGACCAGCAGACTCCT
CGTGAACTACCCGGAGCCGTACAGGTCACAGATCCTCGACTACCTGTTCAAGCCA
AATTTCGGTGCCTCCCTTCATATCCTGAAAGTGGAAATCGGTGGAGATGGACAGA

CTACCGACGGAACGGAGCCCTCCCACATGCATTACGCCCTGGACGAAAATTTCTT
CCGGGGCTACGAGTGGTGGCTGATGAAGGAGGCCAAGAAGCGGAACCCGAACA
TCATCCTGATGGGACTCCCTTGGTCCTTCCCCGGCTGGATCGGAAAGGGATTCAA
CTGGCCCTACGTGAACCTCCAGCTTACCGCCTACTACATCATGACTTGGATTGTG
GGCGCCAAGCATTACCACGACCTGGACATCGACTACATCGGCATTTGGAACGAG
CGGTCCTTTGACATCAACTACATTAAGGTGCTGAGGAGGATGCTGAATTATCAGG
GACTCGACAGAGTGAAGATTATTGCCTCGGACAACCTGTGGGAGCCGATCTCGG
CGTCCATGCTGCTTGATAGCGAGCTCCTCAAGGTCATCGACGTGATCGGAGCCCA
CTACCCTGGTACACACACCGTGAAGGACGCGAAGCTGACCAAGAAGAAGCTGTG
GTCCTCCGAGGACTTCTCCACCCTGAACAGCGATGTCGGAGCCGGATGCTTGGGA
CGGATCCTGAACCAGAACTACGTGAACGGCTACATGACCGCCACCATTGCCTGG
AACCTGGTGGCGTCTTACTATGAGCAACTCCCTTACGGACGCTGTGGGCTGATGA
CTGCCCAGGAACCATGGAGCGGCCACTACGTGGTGGAGTCCCCTATCTGGGTCA
GCGCCCACACCACCCAGTTTACCCAGCCGGGATGGTACTACCTCAAGACCGTGG
GGCACCTTGAGAAGGGAGGATCCTACGTCGCTCTCACTGACGGGCTCGGCAACTT
GACTATCATAGTGGAAACTATGTCCCACAAGCAGTCCGCATGCATTCGGCCCTTC
TTGCCGTACTTCAACGTGTCACGCCAGTTCGCCACTTTCGTGCTGAAGGGTTCGTT
CAGCGAGATCCCGGAGCTCCAAGTCTGGTACACTAAGCTGGGAAAGCCTTCAGA
ACGCTACCTCTTCAAGCAGCTGGACTCCCTGTGGCTGCTGGATTCATCATCGACC
TTCACCCTGGAACTGCAGGAAGATGAAATCTTCACCCTGACCACTCTGACTGTGG
GCAGCAAGGGCTCGTATCCGCTCCCGCCGAAGTCGGAGCCCTTTCCCCAAATCTA
CGAAGATGACTTCGACGTGGACTATCCCTTCTTCTCGGAAGCCCCAAACTTCGCT
GATCAAACCGGAGTGTTTGAGTATTTCACCAACATTGAGGACCCCGGAGAACAC
AGATTCACGCTGCGCCAAGTGCTCAACCAGCGCCCCATCACCTGGGCCGCTGATG
CCTACAACACCATTTCCATCATTGGGGACTACAAATGGTCGAACCTGACCGTGCG
CTGCGACGTGTACATCGAAACCCCCGAAAAGGGCGGCGTGTTCATCGCTGGCCG
GGTCAACAAGGGGGGGATTCTTATTAGATCCGCGAGGGGGATCTTTTTCTGGATC
TTCGCCAACGGGACTTACCGCGTGACCGGAGATCTGGCCGGCTGGGTGATCTACG
CCCTGGGTAGAGTGGACGTGACCGCGAAGAAATGGTACACTCTGACCCTGATTA
TCAAAGGGCGGTTGAGCTCCGGCATGCTGAACGGGAAAACTGTCTGGAAAAACA
TCCCAGTGTCATTCCCTAAGAACGGATGGGCCGCCATCGGAACTCACAGCTTTGA
GTTCGCCCAGTTTGATAACTTTCATGTCGAAGCGACCCGC

Another aspect of the invention relates to an expression cassette comprising a polynucleotide comprising a canine or human GALC open reading frame. In certain embodiments, the polynucleotide is a canine or human codon-optimized sequence, *e.g.,* a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2, or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

The GALC open reading frame in the expression cassette may be operably linked to one or more expression elements that may enhance expression of GALC. In some embodiments, the polynucleotide is operably linked to a promoter, *e.g.,* a chicken beta-actin promoter, *e.g.,* a promoter comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:3 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:3. Chicken beta-actin promoter

TACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCA
CTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAAT
TATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGGG
GCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGC
CAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCG
GCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG

In some embodiments, the polynucleotide is operably linked to a promoter, *e.g.,* a CAGGS promoter, *e.g.,* a promoter comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:4 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:4. CAGGS promoter 1.6kb CMV enhancer, CBA promoter and partial 5' UTR

GATCTGAATTCGGATCTTCAATATTGGCCATTAGCCATATTATTCATTGGTTATAT
AGCATAAATCAATATTGGATATTGGCCATTGCATACGTTGTATCTATATCATAAT
ATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTTGGCATTGATTATT
GACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATG
GAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG
ACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG

GACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCA
GTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTA
AATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTG
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTT
ATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGCGCGCG
CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGC
GGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCG
GCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTG
CGACGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCC
GGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGC
GTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCG
GGGGGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGC
CCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTG
TGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGC
GAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGG
TATGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGC
TGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCT
CGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGC
CGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCC
GGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG
AGAGGGCGCAGGGACTTACTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGA
GGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCA
GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTC
TCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGAC
GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTG
CTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTT
ATTGTGCTGTCTCATCATTTTGGCAAAG

In some embodiments, the polynucleotide is operably linked to a promoter, *e.g.,* a JeT promoter, *e.g.,* a promoter comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:5 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:5. JeT promoter

gggcggagttagggcggagccaatcagcgtgcgccgttccgaaagttgccttttatggctgggcggagaatgggcggtgaacgccg atgattatataaggacgcgccgggtgtggcacagctagttccgtcgcagccgggatttgggtcgcggttcttgtttgt

In some embodiments, the GALC open reading frame is operably linked to a polyadenylation signal, *e.g.,* a synthetic polyadenylation signal, *e.g.,* a polyadenylation signal comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:6 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:6: Synthetic polyadenylation signal (SpA)

AATAAAGAGCTCAGATGCATCGATCAGAGTGTGTTGGTTTTTTGTGTG

In some embodiments, the polynucleotide is operably linked to a polyadenylation signal, *e.g.,* a simian virus 40 (SV40) polyadenylation signal, *e.g.,* a polyadenylation signal comprising, consisting essentially of, or consisting of the nucleotide sequence of SEQ ID NO:7 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:7. SV40 polyadenylation signal (SV40pA)

AGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTG AAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTA TAAGCTGCAATAAACAAGTTAACAACAACAATT

Those skilled in the art will further 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 or foreign and can be a natural or a synthetic sequence. By foreign, 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 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 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), liver specific or preferred, bone marrow specific or preferred, pancreatic specific or preferred, spleen specific or preferred, and lung specific or preferred promoter/enhancer elements. Other inducible promoter/enhancer elements include hormone-inducible and metal-inducible elements. Exemplary inducible promoters/enhancer elements include, but are not limited to, a Tet on/off element, a RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

In embodiments wherein the GALC open reading frame is transcribed and then translated in the target cells, specific initiation signals are generally employed for efficient translation of inserted protein coding sequences. These exogenous translational control sequences, which may include the ATG initiation codon and adjacent sequences, can be of a variety of origins, both natural and synthetic.

In certain embodiments, the expression cassette further comprises at least one adeno-associated virus (AAV) inverted terminal repeat (ITR), *e.g.,* two AAV ITRs. The two ITRs may have the same nucleotide sequence or different nucleotide sequences. The AAV ITRs may be from any AAV serotype, *e.g.,* AAV2, AAV9, AAVrh10, or any AAV serotype as listed in **Table 1**. In some embodiments, the AAV ITRs may be AAV2 ITRs. Each ITR independently may be the wild-type sequence or a modified sequence. In some embodiments, a modified ITR may have a D-element deletion (WO 01/92551). A D-element deletion is defined as the removal of that portion of the ITR known as the D-element. The D-element can be alternatively referred to or known as a D region, or D sequence, and/or the nucleotides of the ITR that do not form palindromic hairpin structures. In some embodiments, the expression cassette is an AAV genome, *e.g.,* a self-complementary AAV genome.

In certain embodiments, the expression cassette comprises a promoter, a human or canine GALC open reading frame, and a polyadenylation site, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV ITR, a promoter, a human

or canine GALC open reading frame, a polyadenylation site, and an AAV ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises a chicken beta actin promoter, a human or canine GALC open reading frame, and an SV40 polyadenylation site, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV ITR, a chicken beta actin promoter, a human or canine GALC open reading frame, an SV40 polyadenylation site, and an AAV ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a chicken beta actin promoter, a human GALC open reading frame, an SV40 polyadenylation site, and an AAV2 ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a chicken beta actin promoter, a canine GALC open reading frame, an SV40 polyadenylation site, and an AAV2 ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV9 ITR, a CAGGS promoter, a human GALC open reading frame, an SV40 polyadenylation site, and an AAV2 ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a CAGGS promoter, a canine GALC open reading frame, an SV40 polyadenylation site, and an AAV2 ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV ITR, a JeT promoter, a human or canine GALC open reading frame, a synthetic polyadenylation site, and an AAV ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a JeT promoter, a human GALC open reading frame, a synthetic polyadenylation site, and an AAV2 ITR, optionally in the recited order. In certain embodiments, the expression cassette comprises an AAV2 ITR, a JeT promoter, a canine GALC open reading frame, a synthetic polyadenylation site, and an AAV2 ITR, optionally in the recited order. The aforementioned components are in operable linkage.

In some embodiments, the expression cassette comprise, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:8 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:8. Human GALC expression cassette with CAGGS/SV40polyA

GGGGGGGGGGGGGGGGGGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG
AGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT

AGATCTGAATTCGGATCTTCAATATTGGCCATTAGCCATATTATTCATTGGTTATA
TAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAAT
ATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTTGGCATTGATTATT
GACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATG
GAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG
ACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGG
GACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCA
GTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGTA
AATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTTG
GCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACG
TTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTT
ATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGCGCGCG
CCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGC
GGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCG
GCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTG
CGACGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCC
GGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCC
TCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGC
GTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCG
GGGGGTGCGTGCGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGC
CCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTG
TGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGC
GAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGG
TATGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGC
TGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCT
CGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGC
CGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCC
GGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG
AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGA
GGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCA
GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTC
TCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGAC
GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTG

CTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGTT
ATTGTGCTGTCTCATCATTTTGGCAAAGAATTCTAGAGGATCCGGTACTCGAGGA
ACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTTTTTGTCTTTTATTTCAGG
TCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTTGCCT
TTACTTCTAGGCCTGTACGGAAGTGTTACTTCTGCTCTAAAAGCTGCGGAATTGT
ACCCGCGGCCCGGGATCCACCGGTGCCACCATGGCTGAGTGGCTCCTTAGCGCG
AGCTGGCAGCGGAGAGCCAAGGCAATGACAGCGGCGGCGGGCTCCGCCGGACG
CGCTGCCGTCCCTCTGTTGCTCTGTGCGTTGCTGGCACCGGGTGGAGCGTATGTG
CTTGATGATTCGGACGGACTCGGTAGAGAATTTGACGGAATCGGAGCGGTCAGC
GGTGGAGGAGCGACGAGCCGCCTGCTCGTGAACTATCCCGAACCCTACCGATCC
CAGATTCTGGACTACCTTTTCAAACCTAACTTCGGCGCAAGCCTTCACATCCTCA
AGGTGGAGATCGGTGGGGACGGTCAGACCACAGACGGTACGGAACCATCGCACA
TGCACTATGCGCTCGACGAAAACTACTTTAGAGGGTATGAGTGGTGGCTGATGA
AAGAGGCCAAAAAGCGGAATCCGAATATCACTCTCATTGGTTTGCCGTGGAGCTT
CCCCGGCTGGCTGGGGAAGGGGTTCGACTGGCCCTATGTGAACCTTCAACTGACA
GCGTATTACGTGGTCACATGGATTGTCGGGGCGAAGAGGTATCATGACTTGGATA
TCGACTATATTGGTATCTGGAACGAGAGATCCTACAACGCAAACTACATCAAAAT
CCTTAGAAAGATGTTGAATTATCAGGGGCTGCAGAGAGTCAAAATCATCGCATC
CGACAATCTTTGGGAATCGATCTCAGCGTCAATGCTCCTCGACGCGGAACTGTTT
AAAGTGGTGGATGTCATTGGGGCGCATTACCCGGGAACACACTCGGCGAAAGAC
GCAAAGTTGACGGGGAAGAAATTGTGGTCGAGCGAGGATTTTTCCACTCTTAATT
CGGATATGGGGGCAGGGTGTTGGGGAAGAATTCTGAACCAGAACTATATCAACG
GGTATATGACCTCGACGATCGCCTGGAATCTTGTGGCATCCTACTACGAGCAGCT
GCCTTACGGGAGGTGCGGTCTTATGACAGCGCAGGAGCCCTGGTCGGGACATTA
CGTCGTCGAGAGCCCCGTATGGGTATCAGCCCACACGACCCAGTTTACACAGCC
GGGCTGGTATTACCTTAAGACGGTGGGCCATCTTGAGAAGGGAGGTAGCTATGT
CGCGCTGACGGATGGCTTGGGTAATTTGACAATCATCATTGAAACTATGTCGCAT
AAACACTCAAAGTGCATTCGCCCTTTTCTGCCCTATTTCAACGTCAGCCAGCAAT
TTGCGACGTTTGTGCTTAAGGGATCGTTTTCGGAGATTCCCGAACTTCAGGTCTG
GTACACGAAACTTGGAAAGACGTCAGAAAGGTTCCTTTTCAAGCAGTTGGACTC
GCTCTGGCTTTTGGATAGCGACGGATCGTTCACTCTGTCCTTGCACGAGGATGAG
TTGTTCACGCTCACTACCCTCACCACTGGCAGAAAGGGCTCCTACCCGTTGCCCC
CGAAAAGCCAGCCGTTTCCTTCAACTTATAAGGATGACTTTAATGTCGATTACCC

ATTCTTCTCGGAGGCCCCGAATTTTGCCGACCAAACAGGAGTATTTGAATACTTC
ACGAACATCGAGGACCCGGGGGAGCACCATTTCACTCTGAGACAAGTGTTGAAC
CAAAGGCCGATTACTTGGGCAGCCGATGCCAGCAATACCATTTCGATTATCGGAG
ACTATAACTGGACAAACTTGACCATCAAATGCGATGTCTATATCGAAACGCCTGA
TACAGGGGGTGTGTTCATCGCTGGTCGCGTAAACAAAGGGGGAATTTTGATCCGC
TCAGCTAGAGGGATCTTCTTTTGGATTTTCGCGAACGGAAGCTACCGCGTGACGG
GAGACTTGGCGGGATGGATCATCTACGCCCTGGGTCGCGTGGAGGTAACAGCGA
AAAAGTGGTACACGTTGACCTTGACAATTAAGGGGCACTTCACGTCCGGGATGCT
GAACGACAAGAGCCTCTGGACGGACATCCCCGTGAATTTCCCCAAAAACGGGTG
GGCAGCAATTGGGACGCACTCCTTTGAATTTGCGCAATTCGACAACTTTTTGGTA
GAGGCTACGCGGTGATAGCCTAGGGATGGCCGCGCGGATCCAGACATGATAAGA
TACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTT
ATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAA
ACAAGTTAACAACAACAATTTAGCAGGCATGCTGGGGAGAGATCTAGGAACCCC
TAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGC
CCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGA
GCGAGCGCGCAGAGAGGGAGTGGCCAACCCCCCCCCCCCCCCC

In some embodiments, the expression cassette comprise, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:9 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:9. Canine GALC expression cassette with CAGGS/SV40polyA

GGGGGGGGGGGGGGGGGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG
AGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAG
TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT
AGATCTGAATTCGGATCTTCAATATTGGCCATTAGCCATATTATTCATTGGTTATA
TAGCATAAATCAATATTGGATATTGGCCATTGCATACGTTGTATCTATATCATAA
TATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTTGGCATTGATTAT
TGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT
GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC
GACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG

GGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACGGT
AAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCAC
GTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATT
TATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGCGCGC
GCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG
CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGC
GGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCT
GCGACGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCC
CGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTC
CTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTG
CGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTC
GGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTG
CCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGT
GTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTG
CGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGG
GTATGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTG
CTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGC
TCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGG
CCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGC
CGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGC
GAGAGGGCGCAGGGACTTACTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGG
AGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGC
AGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTT
CTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGA
CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCT
GCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGGT
TATTGTGCTGTCTCATCATTTTGGCAAAGAATTCTAGAGGATCCGGTACTCGAGG
AACTGAAAAACCAGAAAGTTAACTGGTAAGTTTAGTCTTTTTGTCTTTTATTTCAG
GTCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGATGTTGCC
TTTACTTCTAGGCCTGTACGGAAGTGTTACTTCTGCTCTAAAAGCTGCGGAATTGT
ACCCGCGGCCCGGGATCCAACCGGTGCCACCATGACCGCAGCCGCAGGATCTGC

AGGCCATGCTGCGGTGCCCCTGTTGTTGTGTGCCCTTCTGGTCCCTGGCGGAGCTT
ACGTGCTGGACGACTCCGACGGTTTGGGCCGGGAGTTCGACGGAGTGGGAGCTG
TCTCCGGTGGTGGAGCGACCAGCAGACTCCTCGTGAACTACCCGGAGCCGTACA
GGTCACAGATCCTCGACTACCTGTTCAAGCCAAATTTCGGTGCCTCCCTTCATATC
CTGAAAGTGGAAATCGGTGGAGATGGACAGACTACCGACGGAACGGAGCCCTCC
CACATGCATTACGCCCTGGACGAAAATTTCTTCCGGGGCTACGAGTGGTGGCTGA
TGAAGGAGGCCAAGAAGCGGAACCCGAACATCATCCTGATGGGACTCCCTTGGT
CCTTCCCCCGGCTGGATCGGAAAGGGATTCAACTGGCCCTACGTGAACCTCCAGCT
TACCGCCTACTACATCATGACTTGGATTGTGGGCGCCAAGCATTACCACGACCTG
GACATCGACTACATCGGCATTTGGAACGAGCGGTCCTTTGACATCAACTACATTA
AGGTGCTGAGGAGGATGCTGAATTATCAGGGACTCGACAGAGTGAAGATTATTG
CCTCGGACAACCTGTGGGAGCCGATCTCGGCGTCCATGCTGCTTGATAGCGAGCT
CCTCAAGGTCATCGACGTGATCGGAGCCCACTACCCTGGTACACACACCGTGAA
GGACGCGAAGCTGACCAAGAAGAAGCTGTGGTCCTCCGAGGACTTCTCCACCCT
GAACAGCGATGTCGGAGCCGGATGCTTGGGACGGATCCTGAACCAGAACTACGT
GAACGGCTACATGACCGCCACCATTGCCTGGAACCTGGTGGCGTCTTACTATGAG
CAACTCCCTTACGGACGCTGTGGGCTGATGACTGCCCAGGAACCATGGAGCGGC
CACTACGTGGTGGAGTCCCCTATCTGGGTCAGCGCCCACACCACCCAGTTTACCC
AGCCGGGATGGTACTACCTCAAGACCGTGGGGCACCTTGAGAAGGGAGGATCCT
ACGTCGCTCTCACTGACGGGCTCGGCAACTTGACTATCATAGTGGAAACTATGTC
CCACAAGCAGTCCGCATGCATTCGGCCCTTCTTGCCGTACTTCAACGTGTCACGC
CAGTTCGCCACTTTCGTGCTGAAGGGTTCGTTCAGCGAGATCCCGGAGCTCCAAG
TCTGGTACACTAAGCTGGGAAAGCCTTCAGAACGCTACCTCTTCAAGCAGCTGGA
CTCCCTGTGGCTGCTGGATTCATCATCGACCTTCACCCTGGAACTGCAGGAAGAT
GAAATCTTCACCCTGACCACTCTGACTGTGGGCAGCAAGGGCTCGTATCCGCTCC
CGCCGAAGTCGGAGCCCTTTCCCCAAATCTACGAAGATGACTTCGACGTGGACTA
TCCCTTCTTCTCGGAAGCCCCAAACTTCGCTGATCAAACCGGAGTGTTTGAGTAT
TTCACCAACATTGAGGACCCCGGAGAACACAGATTCACGCTGCGCCAAGTGCTC
AACCAGCGCCCCATCACCTGGGCCGCTGATGCCTACAACACCATTTCCATCATTG
GGGACTACAAATGGTCGAACCTGACCGTGCGCTGCGACGTGTACATCGAAACCC
CCGAAAAGGGCGGCGTGTTCATCGCTGGCCGGGTCAACAAGGGGGGGATTCTTA
TTAGATCCGCGAGGGGGATCTTTTTCTGGATCTTCGCCAACGGGACTTACCGCGT
GACCGGAGATCTGGCCGGCTGGGTGATCTACGCCCTGGGTAGAGTGGACGTGAC

CGCGAAGAAATGGTACACTCTGACCCTGATTATCAAAGGGCGGTTGAGCTCCGG
CATGCTGAACGGGAAAACTGTCTGGAAAAACATCCCAGTGTCATTCCCTAAGAA
CGGATGGGCCGCCATCGGAACTCACAGCTTTGAGTTCGCCCAGTTTGATAACTTT
CATGTCGAAGCGACCCGCTAATGACCTAGGGATGGCCGCGGGGATCCAGACATG
ATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAA
TGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTG
CAATAAACAAGTTAACAACAACAATTTAGCAGGCATGCTGGGGAGAGATCTAGG
AACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGA
GGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGT
GAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACCCCCCCCCCCCCCCCC

In some embodiments, the expression cassette comprise, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:10 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:10. Human GALC expression cassette with JeT/spA

Gggcggagttagggcggagccaatcagcgtgcgccgttccgaaagttgccttttatggctgggcggagaatgggcggtgaacgcc
gatgattatataaggacgcgccgggtgtggcacagctagttccgtcgcagccgggatttgggtcgcggttcttgtttgttccggaaagc
caccATGGCTGAGTGGCTCCTTAGCGCGAGCTGGCAGCGGAGAGCCAAGGCAATG
ACAGCGGCGGCGGGCTCCGCCGGACGCGCTGCCGTCCCTCTGTTGCTCTGTGCGT
TGCTGGCACCGGGTGGAGCGTATGTGCTTGATGATTCGGACGGACTCGGTAGAG
AATTTGACGGAATCGGAGCGGTCAGCGGTGGAGGAGCGACGAGCCGCCTGCTCG
TGAACTATCCCGAACCCTACCGATCCCAGATTCTGGACTACCTTTTCAAACCTAA
CTTCGGCGCAAGCCTTCACATCCTCAAGGTGGAGATCGGTGGGGACGGTCAGAC
CACAGACGGTACGGAACCATCGCACATGCACTATGCGCTCGACGAAAACTACTT
TAGAGGGTATGAGTGGTGGCTGATGAAAGAGGCCAAAAAGCGGAATCCGAATAT
CACTCTCATTGGTTTGCCGTGGAGCTTCCCCGGCTGGCTGGGGAAGGGGTTCGAC
TGGCCCTATGTGAACCTTCAACTGACAGCGTATTACGTGGTCACATGGATTGTCG
GGGCGAAGAGGTATCATGACTTGGATATCGACTATATTGGTATCTGGAACGAGA
GATCCTACAACGCAAACTACATCAAAATCCTTAGAAAGATGTTGAATTATCAGG
GGCTGCAGAGAGTCAAAATCATCGCATCCGACAATCTTTGGGAATCGATCTCAGC
GTCAATGCTCCTCGACGCGGAACTGTTTAAAGTGGTGGATGTCATTGGGGCGCAT

TACCCGGGAACACACTCGGCGAAAGACGCAAAGTTGACGGGGAAGAAATTGTGG
TCGAGCGAGGATTTTTCCACTCTTAATTCGGATATGGGGGCAGGGTGTTGGGGAA
GAATTCTGAACCAGAACTATATCAACGGGTATATGACCTCGACGATCGCCTGGA
ATCTTGTGGCATCCTACTACGAGCAGCTGCCTTACGGGAGGTGCGGTCTTATGAC
AGCGCAGGAGCCCTGGTCGGGACATTACGTCGTCGAGAGCCCCGTATGGGTATC
AGCCCACACGACCCAGTTTACACAGCCGGGCTGGTATTACCTTAAGACGGTGGG
CCATCTTGAGAAGGGAGGTAGCTATGTCGCGCTGACGGATGGCTTGGGTAATTTG
ACAATCATCATTGAAACTATGTCGCATAAACACTCAAAGTGCATTCGCCCTTTTC
TGCCCTATTTCAACGTCAGCCAGCAATTTGCGACGTTTGTGCTTAAGGGATCGTTT
TCGGAGATTCCCGAACTTCAGGTCTGGTACACGAAACTTGGAAAGACGTCAGAA
AGGTTCCTTTTCAAGCAGTTGGACTCGCTCTGGCTTTTGGATAGCGACGGATCGT
TCACTCTGTCCTTGCACGAGGATGAGTTGTTCACGCTCACTACCCTCACCACTGG
CAGAAAGGGCTCCTACCCGTTGCCCCCGAAAAGCCAGCCGTTTCCTTCAACTTAT
AAGGATGACTTTAATGTCGATTACCCATTCTTCTCGGAGGCCCCGAATTTTGCCG
ACCAAACAGGAGTATTTGAATACTTCACGAACATCGAGGACCCGGGGGAGCACC
ATTTCACTCTGAGACAAGTGTTGAACCAAAGGCCGATTACTTGGGCAGCCGATGC
CAGCAATACCATTTCGATTATCGGAGACTATAACTGGACAAACTTGACCATCAAA
TGCGATGTCTATATCGAAACGCCTGATACAGGGGGTGTGTTCATCGCTGGTCGCG
TAAACAAAGGGGGAATTTTGATCCGCTCAGCTAGAGGGATCTTCTTTTGGATTTT
CGCGAACGGAAGCTACCGCGTGACGGGAGACTTGGCGGGATGGATCATCTACGC
CCTGGGTCGCGTGGAGGTAACAGCGAAAAAGTGGTACACGTTGACCTTGACAAT
TAAGGGGCACTTCACGTCCGGGATGCTGAACGACAAGAGCCTCTGGACGGACAT
CCCCGTGAATTTCCCCAAAAACGGGTGGGCAGCAATTGGGACGCACTCCTTTGAA
TTTGCGCAATTCGACAACTTTTTGGTAGAGGCTACGCGGaggcctAATAAAGAGCTC
AGATGCATCGATCAGAGTGTGTTGGTTTTTTGTGTG

In some embodiments, the expression cassette comprise, consists essentially of, or consists of the nucleotide sequence of SEQ ID NO:11 or a sequence at least about 70% identical thereto, *e.g.,* at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical thereto.

SEQ ID NO:11. Canine GALC expression cassette with JeT/spA

gggcggagttagggcggagccaatcagcgtgcgccgttccgaaagttgccttttatggctgggcggagaatgggcggtgaacgccg
atgattatataaggacgcgccgggtgtggcacagctagttccgtcgcagccgggatttgggtcgcggttcttgtttgttccggaaagcc
accATGACCGCAGCCGCAGGATCTGCAGGCCATGCTGCGGTGCCCCTGTTGTTGTG
TGCCCTTCTGGTCCCTGGCGGAGCTTACGTGCTGGACGACTCCGACGGTTTGGGC
CGGGAGTTCGACGGAGTGGGAGCTGTCTCCGGTGGTGGAGCGACCAGCAGACTC
CTCGTGAACTACCCGGAGCCGTACAGGTCACAGATCCTCGACTACCTGTTCAAGC
CAAATTTCGGTGCCTCCCTTCATATCCTGAAAGTGGAAATCGGTGGAGATGGACA
GACTACCGACGGAACGGAGCCCTCCCACATGCATTACGCCCTGGACGAAAATTT
CTTCCGGGGCTACGAGTGGTGGCTGATGAAGGAGGCCAAGAAGCGGAACCCGAA
CATCATCCTGATGGGACTCCCTTGGTCCTTCCCCGGCTGGATCGGAAAGGGATTC
AACTGGCCCTACGTGAACCTCCAGCTTACCGCCTACTACATCATGACTTGGATTG
TGGGCGCCAAGCATTACCACGACCTGGACATCGACTACATCGGCATTTGGAACG
AGCGGTCCTTTGACATCAACTACATTAAGGTGCTGAGGAGGATGCTGAATTATCA
GGGACTCGACAGAGTGAAGATTATTGCCTCGGACAACCTGTGGGAGCCGATCTC
GGCGTCCATGCTGCTTGATAGCGAGCTCCTCAAGGTCATCGACGTGATCGGAGCC
CACTACCCTGGTACACACACCGTGAAGGACGCGAAGCTGACCAAGAAGAAGCTG
TGGTCCTCCGAGGACTTCTCCACCCTGAACAGCGATGTCGGAGCCGGATGCTTGG
GACGGATCCTGAACCAGAACTACGTGAACGGCTACATGACCGCCACCATTGCCT
GGAACCTGGTGGCGTCTTACTATGAGCAACTCCCTTACGGACGCTGTGGGCTGAT
GACTGCCCAGGAACCATGGAGCGGCCACTACGTGGTGGAGTCCCCTATCTGGGT
CAGCGCCCACACCACCCAGTTTACCCAGCCGGGATGGTACTACCTCAAGACCGT
GGGGCACCTTGAGAAGGGAGGATCCTACGTCGCTCTCACTGACGGGCTCGGCAA
CTTGACTATCATAGTGGAAACTATGTCCCACAAGCAGTCCGCATGCATTCGGCCC
TTCTTGCCGTACTTCAACGTGTCACGCCAGTTCGCCACTTTCGTGCTGAAGGGTTC
GTTCAGCGAGATCCCGGAGCTCCAAGTCTGGTACACTAAGCTGGGAAAGCCTTC
AGAACGCTACCTCTTCAAGCAGCTGGACTCCCTGTGGCTGCTGGATTCATCATCG
ACCTTCACCCTGGAACTGCAGGAAGATGAAATCTTCACCCTGACCACTCTGACTG
TGGGCAGCAAGGGCTCGTATCCGCTCCCGCCGAAGTCGGAGCCCTTTCCCCAAAT
CTACGAAGATGACTTCGACGTGGACTATCCCTTCTTCTCGGAAGCCCCAAACTTC
GCTGATCAAACCGGAGTGTTTGAGTATTTCACCAACATTGAGGACCCCGGAGAA
CACAGATTCACGCTGCGCCAAGTGCTCAACCAGCGCCCCATCACCTGGGCCGCTG
ATGCCTACAACACCATTTCCATCATTGGGGACTACAAATGGTCGAACCTGACCGT

GCGCTGCGACGTGTACATCGAAACCCCCGAAAAGGGCGGCGTGTTCATCGCTGG
CCGGGTCAACAAGGGGGGGATTCTTATTAGATCCGCGAGGGGGATCTTTTTCTGG
ATCTTCGCCAACGGGACTTACCGCGTGACCGGAGATCTGGCCGGCTGGGTGATCT
ACGCCCTGGGTAGAGTGGACGTGACCGCGAAGAAATGGTACACTCTGACCCTGA
TTATCAAAGGGCGGTTGAGCTCCGGCATGCTGAACGGGAAAACTGTCTGGAAAA
ACATCCCAGTGTCATTCCCTAAGAACGGATGGGCCGCCATCGGAACTCACAGCTT
TGAGTTCGCCCAGTTTGATAACTTTCATGTCGAAGCGACCCGCaggcctAATAAAGA
GCTCAGATGCATCGATCAGAGTGTGTTGGTTTTTTGTGTG

A further aspect of the invention relates to a vector comprising the polynucleotide or the expression cassette of the invention. Suitable vectors include, but are not limited to, a plasmid, phage, viral vector (*e.g.,* an AAV vector, an adenovirus vector, a herpesvirus vector, an alphavirus vector, or a baculovirus vector), bacterial artificial chromosome (BAC), or yeast artificial chromosome (YAC). For example, the nucleic acid can comprise, consist of, or consist essentially of an AAV vector comprising a 5' and/or 3' terminal repeat (*e.g.,* 5' and/or 3' AAV terminal repeat). In some embodiments, the vector is a delivery vehicle such as a particle (*e.g.,* a microparticle or nanoparticle) or a liposome to which the expression cassette is attached or in which the expression cassette is embedded. The vector may be any delivery vehicle suitable to carry the expression cassette into a cell.

In some embodiments, the vector is a viral vector, *e.g.,* an AAV vector. The AAV vector may be any AAV serotype, *e.g.,* AAV9, AAVrh10, AAVOlig100, or any AAV serotype as listed in **Table 1**. In some embodiments, the AAV vector may comprise wild-type capsid proteins. In other embodiments, the AAV vector may comprise a modified capsid protein with altered tropism compared to a wild-type capsid protein, *e.g.,* a modified capsid protein is liver-detargeted or has enhanced tropism for particular cells.

In some embodiments, the vector is a single-stranded AAV (ssAAV) vector. In some embodiments, the vector is a self-complementary or duplexed AAV (scAAV) vector. scAAV vectors are described in international patent publication WO 01/92551 (the disclosure of which is incorporated herein by reference in its entirety). Use of scAAV to express the GALC ORF may provide an increase in the number of cells transduced, the copy number per transduced cell, or both.

An additional aspect of the invention relates to a transformed cell comprising the polynucleotide, expression cassette, and/or vector of the invention. In some embodiments, the

polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome. The cell may be an *in vitro*, *ex vivo*, or *in vivo* cell.

Another aspect of the invention relates to a transgenic animal comprising the polynucleotide, expression cassette, vector, and/or the transformed cell of the invention. In some embodiments, the animal is a laboratory animal, *e.g.*, a mouse, rat, rabbit, dog, monkey, or non-human primate.

A further aspect of the invention relates to a pharmaceutical formulation comprising the polynucleotide, expression cassette, vector, and/or transformed cell of the invention in a pharmaceutically acceptable carrier.

In a specific embodiment, the polynucleotide, expression cassette, vector, and/or transformed cell of the invention is isolated.

In another specific embodiment, the polynucleotide, expression cassette, vector, and/or transformed cell of the invention is purified.

Methods of Producing Virus Vectors

The present invention further provides methods of producing virus vectors. In one particular embodiment, the present invention provides a method of producing a recombinant AAV particle, comprising providing to a cell permissive for AAV replication: (a) a recombinant AAV template comprising (i) the polynucleotide or expression cassette of the invention, and (ii) an ITR; (b) a polynucleotide comprising Rep coding sequences and Cap coding sequences; under conditions sufficient for the replication and packaging of the recombinant AAV template; whereby recombinant AAV particles are produced in the cell. Conditions sufficient for the replication and packaging of the recombinant AAV template can be, *e.g.*, the presence of AAV sequences sufficient for replication of the AAV template and encapsidation into AAV capsids (*e.g.*, AAV *rep* sequences and AAV *cap* sequences) and helper sequences from adenovirus and/or herpesvirus. In particular embodiments, the AAV template comprises two AAV ITR sequences, which are located 5' and 3' to the polynucleotide of the invention, although they need not be directly contiguous thereto.

In some embodiments, the recombinant AAV template comprises an ITR that is not resolved by Rep to make duplexed AAV vectors as described in international patent publication WO 01/92551.

The AAV template and AAV *rep* and *cap* sequences are provided under conditions such that virus vector comprising the AAV template packaged within the AAV capsid is

produced in the cell. The method can further comprise the step of collecting the virus vector from the cell. The virus vector can be collected from the medium and/or by lysing the cells.

The cell can be a cell that is permissive for AAV viral replication. Any suitable cell known in the art may be employed. In particular embodiments, the cell is a mammalian cell (*e.g.,* a primate, canine, or human cell). As another option, the cell can be a trans-complementing packaging cell line that provides functions deleted from a replication-defective helper virus, *e.g.,* 293 cells or other E1a trans-complementing cells.

The AAV replication and capsid sequences may be provided by any method known in the art. Current protocols typically express the AAV *rep/cap* genes on a single plasmid. The AAV replication and packaging sequences need not be provided together, although it may be convenient to do so. The AAV *rep* and/or *cap* sequences may be provided by any viral or non-viral vector. For example, the *rep/cap* sequences may be provided by a hybrid adenovirus or herpesvirus vector (*e.g.,* inserted into the E1a or E3 regions of a deleted adenovirus vector). EBV vectors may also be employed to express the AAV *cap* and *rep* genes. One advantage of this method is that EBV vectors are episomal, yet will maintain a high copy number throughout successive cell divisions (*i.e.,* are stably integrated into the cell as extra-chromosomal elements, designated as an "EBV based nuclear episome," *see* Margolski, (1992) *Curr. Top. Microbiol. Immun.* 158:67).

As a further alternative, the *rep/cap* sequences may be stably incorporated into a cell.

Typically the AAV *rep/cap* sequences will not be flanked by the TRs, to prevent rescue and/or packaging of these sequences.

The AAV template can be provided to the cell using any method known in the art. For example, the template can be supplied by a non-viral (*e.g.,* plasmid) or viral vector. In particular embodiments, the AAV template is supplied by a herpesvirus or adenovirus vector (*e.g.,* inserted into the E1a or E3 regions of a deleted adenovirus). As another illustration, Palombo *et al.*, (1998) *J. Virology* 72:5025, describes a baculovirus vector carrying a reporter gene flanked by the AAV TRs. EBV vectors may also be employed to deliver the template, as described above with respect to the *rep/cap* genes.

In another representative embodiment, the AAV template is provided by a replicating rAAV virus. In still other embodiments, an AAV provirus comprising the AAV template is stably integrated into the chromosome of the cell.

To enhance virus titers, helper virus functions (*e.g.,* adenovirus or herpesvirus) that promote a productive AAV infection can be provided to the cell. Helper virus sequences

necessary for AAV replication are known in the art. Typically, these sequences will be provided by a helper adenovirus or herpesvirus vector. Alternatively, the adenovirus or herpesvirus sequences can be provided by another non-viral or viral vector, *e.g.,* as a non-infectious adenovirus miniplasmid that carries all of the helper genes that promote efficient AAV production as described by Ferrari *et al.*, (1997) *Nature Med.* 3:1295, and U.S. Patent Nos. 6,040,183 and 6,093,570.

Further, the helper virus functions may be provided by a packaging cell with the helper sequences embedded in the chromosome or maintained as a stable extrachromosomal element. Generally, the helper virus sequences cannot be packaged into AAV virions, *e.g.,* are not flanked by ITRs.

Those skilled in the art will appreciate that it may be advantageous to provide the AAV replication and capsid sequences and the helper virus sequences (*e.g.,* adenovirus sequences) on a single helper construct. This helper construct may be a non-viral or viral construct. As one nonlimiting illustration, the helper construct can be a hybrid adenovirus or hybrid herpesvirus comprising the AAV *rep/cap* genes.

In one particular embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. This vector can further comprise the AAV template. The AAV *rep/cap* sequences and/or the AAV template can be inserted into a deleted region (*e.g.,* the E1a or E3 regions) of the adenovirus.

In a further embodiment, the AAV *rep/cap* sequences and the adenovirus helper sequences are supplied by a single adenovirus helper vector. According to this embodiment, the AAV template can be provided as a plasmid template.

In another illustrative embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper vector, and the AAV template is integrated into the cell as a provirus. Alternatively, the AAV template is provided by an EBV vector that is maintained within the cell as an extrachromosomal element (*e.g.,* as an EBV based nuclear episome).

In a further exemplary embodiment, the AAV *rep/cap* sequences and adenovirus helper sequences are provided by a single adenovirus helper. The AAV template can be provided as a separate replicating viral vector. For example, the AAV template can be provided by an AAV particle or a second recombinant adenovirus particle.

According to the foregoing methods, the hybrid adenovirus vector typically comprises the adenovirus 5' and 3' *cis* sequences sufficient for adenovirus replication and packaging

(*i.e.,* the adenovirus terminal repeats and PAC sequence). The AAV *rep/cap* sequences and, if present, the AAV template are embedded in the adenovirus backbone and are flanked by the 5' and 3' *cis* sequences, so that these sequences may be packaged into adenovirus capsids. As described above, the adenovirus helper sequences and the AAV *rep/cap* sequences are generally not flanked by ITRs so that these sequences are not packaged into the AAV virions.

Zhang *et al.*, ((2001) *Gene Ther.* 18:704-12) describe a chimeric helper comprising both adenovirus and the AAV *rep* and *cap* genes.

Herpesvirus may also be used as a helper virus in AAV packaging methods. Hybrid herpesviruses encoding the AAV Rep protein(s) may advantageously facilitate scalable AAV vector production schemes. A hybrid herpes simplex virus type I (HSV-1) vector expressing the AAV-2 *rep* and *cap* genes has been described (Conway *et al.*, (1999) *Gene Ther.* 6:986 and WO 00/17377).

As a further alternative, the virus vectors of the invention can be produced in insect cells using baculovirus vectors to deliver the *rep/cap* genes and AAV template as described, for example, by Urabe *et al.*, (2002) *Human Gene Ther.* 13:1935-43.

AAV vector stocks free of contaminating helper virus may be obtained by any method known in the art. For example, AAV and helper virus may be readily differentiated based on size. AAV may also be separated away from helper virus based on affinity for a heparin substrate (Zolotukhin *et al.* (1999) *Gene Therapy* 6:973). Deleted replication-defective helper viruses can be used so that any contaminating helper virus is not replication competent. As a further alternative, an adenovirus helper lacking late gene expression may be employed, as only adenovirus early gene expression is required to mediate packaging of AAV. Adenovirus mutants defective for late gene expression are known in the art (*e.g.,* ts100K and ts149 adenovirus mutants).

Methods of Using GALC Vectors

The present invention also relates to methods for delivering a GALC ORF to a cell or a subject to increase production of GALC, *e.g.,* for therapeutic or research purposes *in vitro*, *ex vivo*, or *in vivo*. Thus, one aspect of the invention relates to a method of expressing a GALC open reading frame in a cell, comprising contacting the cell with the polynucleotide, expression cassette, and/or the vector of the invention, thereby expressing the GALC open reading frame in the cell. In some embodiments, the cell is an *in vitro* cell, an *ex vivo* cell, or an *in vivo* cell.

Another aspect of the invention relates to a method of expressing a GALC open reading frame in a subject, comprising delivering to the subject the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, thereby expressing the GALC open reading frame in the subject. In some embodiments, the subject is an animal model of a disorder associated with aberrant GALC gene expression.

The invention further provides a method of treating a disorder associated with aberrant expression of a GALC gene or aberrant activity of a GALC gene product in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of the polynucleotide, expression cassette, vector, and/or transformed cell of the invention, thereby treating the disorder associated with aberrant expression of the GALC gene or aberrant activity of a GALC gene product in the subject. The invention provides a method of treating a disorder associated with aberrant expression of a GALC gene or aberrant activity of a GALC gene product in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, the expression cassette, vector, and/or transformed cell of the invention, such that the GALC open reading frame is expressed in the subject. In some embodiments, the disorder associated with expression of the GALC gene or gene product is Krabbe disease (*i.e.,* globoid cell leukodystrophy).

The invention further provides a method of treating Krabbe disease (*i.e.,* globoid cell leukodystrophy) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide, the expression cassette, vector, and/or transformed cell of the invention, such that the GALC open reading frame is expressed in the subject.

In some embodiments, the methods of the present invention further comprise administering to the subject a bone marrow transplant (BMT), *e.g.,* prior to administering the effective amount of a polynucleotide, expression cassette, vector, and/or transformed cell of the present invention. Techniques for performing BMT (referred to interchangeably as a hematopoietic stem cell transplant (HSCT)) are well known to those of skill in the art, and are routine for clinicians in the treatment of subjects (*e.g.,* patients, *e.g.,* canine and/or human patients) in need thereof. The skilled clinician can readily determine the proper regimen to be used for performing BMT based on factors including the age and condition of the subject, type of disease being treated, stage of the disease, patient size, and the like.

In certain embodiments, the polynucleotide, expression cassette, vector, and/or transformed cell is delivered to the subject, *e.g.,* systemically (*e.g.,* intravenously) or directly

to the central nervous system (*e.g.,* to the cerebrospinal fluid by intrathecal or intraventricular injection) of the subject. In some embodiments, the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intravenously. In some embodiments, the polynucleotide, expression cassette, vector, and/or transformed cell is delivered intracerebroventricularly.

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), cattle, sheep, goats, pigs, horses, cats, dogs, rabbits, rodents (*e.g.,* rats, mice, hamsters, and the like), *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. As a further option, the subject can be a laboratory animal and/or an animal model of disease. Preferably, the subject is a canine or human.

In certain embodiments, the polynucleotide of the invention is administered to a subject in need thereof as early as possible in the life of the subject, *e.g.,* as soon as the subject is diagnosed with aberrant GALC expression or activity or any of the above-mentioned diseases or disorders. In some embodiments, the polynucleotide is administered to a newborn subject, *e.g.,* after newborn screening has identified aberrant GALC expression or activity. In some embodiments, the polynucleotide is administered to a fetus *in utero*, *e.g.,* after prenatal screening has identified aberrant GALC expression or activity or the presence of one of the above-mentioned diseases or disorders. In some embodiments, the polynucleotide is administered to a subject as soon as the subject develops symptoms associated with aberrant GALC expression or activity or is suspected or diagnosed as having aberrant GALC expression or activity or one of the above-mentioned diseases or disorders. In some embodiments, the polynucleotide is administered to a subject before the subject develops symptoms associated with aberrant GALC expression or activity or disease/disorder, *e.g.,* a subject that is suspected or diagnosed as having aberrant GALC expression or activity or one of the above-mentioned diseases or disorders but has not started to exhibit symptoms.

In particular embodiments, the present invention provides a pharmaceutical composition comprising a polynucleotide, expression cassette, vector, and/or transformed cell 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.

One aspect of the present invention is a method of transferring a GALC ORF to a cell *in vitro*. The polynucleotide, expression cassette, and/or vector of the invention 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 10^5 infectious units are introduced to the cell.

The 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, liver stem cell). As still a further

alternative, the cell may be a cancer or tumor cell. Moreover, the cells can be from any species of origin, as indicated above.

The polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, 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, the polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, is 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 polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, is 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 *ex vivo* are administered to the subject in an effective amount in combination with a pharmaceutical carrier.

A further aspect of the invention is a method of administering the polynucleotide, expression cassette, and/or vector of the invention, *e.g.,* virus vector, to a subject. In particular embodiments, the method comprises a method of delivering a GALC ORF to an animal subject, the method comprising: administering an effective amount of a virus vector according to the invention to an animal subject. 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. Optionally, the virus vector is delivered in an effective dose in a pharmaceutically acceptable carrier.

Dosages of the virus vectors to be administered to a subject will depend upon the mode of administration, the disease or condition to be treated, the individual subject's condition, the particular virus vector, and the nucleic acid to be delivered, and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are virus titers of at least about 10^2, 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}, 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, yet more preferably about 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, or 10^{15} transducing units. In some embodiments, the dose of total virus may be in the range of about $1x10^{10}$, $1.5x10^{10}$, $2x10^{10}$, $2.5x10^{10}$, $3x10^{10}$, $3.5x10^{10}$, $4x10^{10}$, $4.5x10^{10}$, $5x10^{10}$, $5.5x10^{10}$, $6x10^{10}$, $6.5x10^{10}$, $7x10^{10}$, $7.5x10^{10}$, $8x10^{10}$, $8.5x10^{10}$, $9x10^{10}$, $9.5x10^{10}$, $1x10^{11}$, $1.5x10^{11}$, $2x10^{11}$, $2.5x10^{11}$, $3x10^{11}$, $3.5x10^{11}$, $4x10^{11}$, $4.5x10^{11}$, $5x10^{11}$, $5.5x10^{11}$, $6x10^{11}$, $6.5x10^{11}$, $7x10^{11}$, $7.5x10^{11}$, $8x10^{11}$, $8.5x10^{11}$, $9x10^{11}$, $9.5x10^{11}$, $1x10^{12}$, $1.5x10^{12}$, $2x10^{12}$, $2.5x10^{12}$, $3x10^{12}$, $3.5x10^{12}$, $4x10^{12}$, $4.5x10^{12}$, $5x10^{12}$, $5.5x10^{12}$, $6x10^{12}$, $6.5x10^{12}$, $7x10^{12}$, $7.5x10^{12}$, $8x10^{12}$, $8.5x10^{12}$, $9x10^{12}$, $9.5x10^{12}$, $1x10^{13}$, $1.5x10^{13}$, $2x10^{13}$, $2.5x10^{13}$, $3x10^{13}$, $3.5x10^{13}$, $4x10^{13}$, $4.5x10^{13}$, $5x10^{13}$, $5.5x10^{13}$, $6x10^{13}$, $6.5x10^{13}$, $7x10^{13}$, $7.5x10^{13}$, $8x10^{13}$, $8.5x10^{13}$, $9x10^{13}$, $9.5x10^{13}$, $1x10^{14}$, $1.5x10^{14}$, $2x10^{14}$, $2.5x10^{14}$, $3x10^{14}$, $3.5x10^{14}$, $4x10^{14}$, $4.5x10^{14}$, $5x10^{14}$, $5.5x10^{14}$, $6x10^{14}$, $6.5x10^{14}$, $7x10^{14}$, $7.5x10^{14}$, $8x10^{14}$, $8.5x10^{14}$, $9x10^{14}$, $9.5x10^{14}$, $1x10^{15}$, $1.5x10^{15}$, $2x10^{15}$, $2.5x10^{15}$, $3x10^{15}$, $3.5x10^{15}$, $4x10^{15}$, $4.5x10^{15}$, $5x10^{15}$, $5.5x10^{15}$, $6x10^{15}$, $6.5x10^{15}$, $7x10^{15}$, $7.5x10^{15}$, $8x10^{15}$, $8.5x10^{15}$, $9x10^{15}$, $9.5x10^{15}$, $1x10^{16}$, $1.5x10^{16}$, $2x10^{16}$, $2.5x10^{16}$, $3x10^{16}$, $3.5x10^{16}$, $4x10^{16}$, $4.5x10^{16}$, $5x10^{16}$, $5.5x10^{16}$, $6x10^{16}$, $6.5x10^{16}$, $7x10^{16}$, $7.5x10^{16}$, $8x10^{16}$, $8.5x10^{16}$, $9x10^{16}$, $9.5x10^{16}$, or any value or range therein. In some embodiments, the dose of total virus may be in the range of about $2x10^{11}$ to about $7.7x10^{13}$. In some embodiments, the dose of total virus may be in the range of about $6.6x10^{11}$ to about $3.5x10^{12}$. In some embodiments, the dose of total virus may be in the range of about $1.4x10^{10}$ to about $3.4x10^{16}$. In some embodiments, the dose of virus per gram of actual or estimated brain weight may be in the range of about $1x10^{10}$, $1.5x10^{10}$, $2x10^{10}$, $2.5x10^{10}$, $3x10^{10}$, $3.5x10^{10}$, $4x10^{10}$, $4.5x10^{10}$, $5x10^{10}$, $5.5x10^{10}$, $6x10^{10}$, $6.5x10^{10}$, $7x10^{10}$, $7.5x10^{10}$, $8x10^{10}$, $8.5x10^{10}$, $9x10^{10}$, $9.5x10^{10}$, $1x10^{11}$, $1.5x10^{11}$, $2x10^{11}$, $2.5x10^{11}$, $3x10^{11}$, $3.5x10^{11}$, $4x10^{11}$, $4.5x10^{11}$, $5x10^{11}$, $5.5x10^{11}$, $6x10^{11}$, $6.5x10^{11}$, $7x10^{11}$, $7.5x10^{11}$, $8x10^{11}$, $8.5x10^{11}$, $9x10^{11}$, $9.5x10^{11}$, $1x10^{12}$, $1.5x10^{12}$, $2x10^{12}$, $2.5x10^{12}$, $3x10^{12}$, $3.5x10^{12}$, $4x10^{12}$, $4.5x10^{12}$, $5x10^{12}$, $5.5x10^{12}$, $6x10^{12}$, $6.5x10^{12}$, $7x10^{12}$, $7.5x10^{12}$, $8x10^{12}$, $8.5x10^{12}$, $9x10^{12}$, $9.5x10^{12}$, $1x10^{13}$, $1.5x10^{13}$, $2x10^{13}$, $2.5x10^{13}$, $3x10^{13}$, $3.5x10^{13}$, $4x10^{13}$, $4.5x10^{13}$, $5x10^{13}$, $5.5x10^{13}$, $6x10^{13}$, $6.5x10^{13}$, $7x10^{13}$, $7.5x10^{13}$, $8x10^{13}$, $8.5x10^{13}$, $9x10^{13}$, $9.5x10^{13}$, $1x10^{14}$, $1.5x10^{14}$, $2x10^{14}$, $2.5x10^{14}$, $3x10^{14}$, $3.5x10^{14}$, $4x10^{14}$, $4.5x10^{14}$, $5x10^{14}$, $5.5x10^{14}$, $6x10^{14}$, $6.5x10^{14}$, $7x10^{14}$, $7.5x10^{14}$, $8x10^{14}$, $8.5x10^{14}$, $9x10^{14}$, $9.5x10^{14}$, $1x10^{15}$, $1.5x10^{15}$, $2x10^{15}$, $2.5x10^{15}$, $3x10^{15}$, $3.5x10^{15}$, $4x10^{15}$, $4.5x10^{15}$, $5x10^{15}$, $5.5x10^{15}$, $6x10^{15}$, $6.5x10^{15}$, $7x10^{15}$, $7.5x10^{15}$, $8x10^{15}$, $8.5x10^{15}$, $9x10^{15}$, $9.5x10^{15}$, $1x10^{16}$, $1.5x10^{16}$, $2x10^{16}$, $2.5x10^{16}$, $3x10^{16}$, $3.5x10^{16}$, $4x10^{16}$, $4.5x10^{16}$, $5x10^{16}$, $5.5x10^{16}$, $6x10^{16}$, $6.5x10^{16}$, $7x10^{16}$, $7.5x10^{16}$, $8x10^{16}$, $8.5x10^{16}$, $9x10^{16}$, $9.5x10^{16}$, or any value or range therein. In some embodiments, the dose of virus per actual or

estimated brain weight may be in the range of about $2x10^{11}$ to about $7.7x10^{13}$. In some embodiments, the dose of virus per actual or estimated brain weight may be in the range of about $6.6x10^{11}$ to about $3.5x10^{12}$. In some embodiments, the dose of virus per actual or estimated brain weight may be in the range of about $1.4x10^{10}$ to about $3.4x10^{16}$. Doses and virus titer transducing units may be calculated as vector or viral genomes (vg).

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.*

Exemplary modes of administration include oral, rectal, transmucosal, topical, intranasal, inhalation (*e.g.,* via an aerosol), buccal (*e.g.,* sublingual), vaginal, intrathecal, intraocular, transdermal, *in utero* (or *in ovo*), parenteral (*e.g.,* intravenous, subcutaneous, intradermal, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intradermal, intrapleural, intracerebral, and intraarticular), topical (*e.g.,* to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intro-lymphatic, and the like, as well as direct tissue or organ injection (*e.g.,* to liver, skeletal muscle, cardiac muscle, diaphragm muscle or brain). Administration can also be to a tumor (*e.g.,* in or a near a tumor or a lymph node). 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 some embodiments, the viral vector is administered to the CNS, the peripheral nervous system, or both.

In some embodiments, the viral vector is administered directly to the CNS, *e.g.,* the brain or the spinal cord. Direct administration can result in high specificity of transduction of CNS cells, *e.g.,* wherein at least 80%, 85%, 90%, 95% or more of the transduced cells are CNS cells. Any method known in the art to administer vectors directly to the CNS can be used. The vector may be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes, cortex, basal ganglia, hippocampus and amygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus. The vector may also be administered to different regions of the eye such as the retina, cornea or optic nerve. The vector may be delivered into the cerebrospinal fluid (*e.g.,* by lumbar puncture) for more disperse administration of the vector.

The delivery vector may be administered to the desired region(s) of the CNS by any route known in the art, including but not limited to, intrathecal, intracerebral, intraventricular, intranasal, intra-aural, intra-ocular (*e.g.,* intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (*e.g.,* sub-Tenon's region) delivery or any combination thereof.

The delivery vector may be administered in a manner that produces a more widespread, diffuse transduction of tissues, including the CNS, the peripheral nervous system, and/or other tissues.

Typically, the viral vector will be administered in a liquid formulation by direct injection (*e.g.,* stereotactic injection) to the desired region or compartment in the CNS and/or other tissues. In some embodiments, the vector can be delivered via a reservoir and/or pump. In other embodiments, the vector may be provided by topical application to the desired region or by intra-nasal administration of an aerosol formulation. Administration to the eye or into the ear may be by topical application of liquid droplets. As a further alternative, the vector may be administered as a solid, slow-release formulation. Controlled release of parvovirus and AAV vectors is described by international patent publication WO 01/91803.

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: Similarities between rodent, macaque, canine, and human GALC

Gene therapy intervention for infantile Krabbe disease is proposed with the following advantages: the procedure is relatively safer and requires a shorter hospitalization, provides GALC enzyme to the tissue faster, sustained gene expression that lasts for a longer duration, provides better tissue distribution with potentially improved clinical benefits to the patients and thus expected to be more effective than HSCT in improving the quality of life and event-free survival. Treatment can be performed, *e.g.,* with AAV9/GALC, with a codon-optimized GALC ORF for use in mice, rats, dogs, and humans. Similarities between the optimized human *hGALCopt* and wildtype GALC sequences in rodents, macaques, and dogs are shown in **FIG. 1**.

AAV9/GALC is a recombinant serotype 9 adeno-associated virus (AAV) encoding a human *GALC* transgene (*hGALCopt*). The codons are optimized to improve expression without any changes to the amino acid sequence. The final product consists of AAV9 capsids that are packaged with the single-stranded AAV genome comprising an AAV2 inverted terminal repeat (ITR), the "CAGGS" version of the "CBA" promoter (1.6 kb total CMV enhancer, chicken beta actin promoter and partial 5' untranslated region), codon-optimized human GALC DNA coding sequence, SV40 polyadenylation signal, and WT AAV2 ITR. Codon-optimization improves expression of the protein, but does not change the amino acid sequence.

EXAMPLE 2: GALC delivery in neonatal mice improved survival.

A spontaneous autosomal recessive mutation observed in a mouse colony at the Jackson Laboratory was characterized and identified as twitcher (*twi*) displaying ataxia (twitching). Further studies in these mice established them as a relevant model of the human disorder (Kobayashi et al. 1980 Brain Res. 202:479-483; Moser 2006 Neurology 67(2):201-202). The twitcher mice have a complete loss of GALC activity and an accompanying accumulation of psychosine. On a pure C57Bl/6 background the reported median survival of twitcher mice is around 42 days.

In this study, neonatal (P0-P1) GALC$^{-/-}$ "twitcher" mice received the murine AAV9/mGALC via multiple routes of administration simultaneously (intracranial, intrathecal, and intravenous) at a total dose of 4.2x10^{11} vg per mouse. Treated mice had a median survival of 263 days (maximum 484 days) compared to untreated GALC$^{-/-}$ cohort that had a median survival of 44 days (maximum 47 days) (Marshall et al. 2018 Mol. Ther. 26(3):874-889). Analysis done at P40 evaluated the therapy compared to untreated mice. Since all the untreated mice died by P47 all analyses done in treated mice that aged beyond that point were compared to wild type mice. The treated GALC$^{-/-}$ cohort had improved clinical scoring, body weight, motor function, endurance and general locomotion compared to untreated cohorts at P40 testing. On some of the functional assays the performance of the treated GALC$^{-/-}$ mice was comparable to wild type cohorts during P100-P200 time points. Gene therapy significantly improved GALC activity and concurrently reduced the psychosine accumulation in the brain, spinal cord and sciatic nerve at P40. This trend was maintained in treated mice until they aged to humane endpoint of either limb paralysis or predetermined rate of weight loss.

EXAMPLE 3: GALC delivery in PND11 mice improved histology and survival.

GALC$^{-/-}$ "twitcher" mice received a single intrathecal injection of AAV9/mGALC into the lumbar cistern at PND11, and then they were monitored for survival and biochemical/histological improvement relative to untreated control mice (Karumuthil-Melethil et al. 2016 J. Neurosci. Res. 94(11):1138-1151). Survival of the treated mice was extended 79 days when receiving a dose of 2x10^{11} vg total where untreated controls were dead by 40 days. Death in these cohorts was a predefined humane endpoint set to a loss in 20% body weight. A separate group of mice were sacrificed at PND35 for investigation of vector biodistribution, GALC activity, psychosine levels, and histological myelination improvements (**Figure 2**). These data were all performed at the dose of 2x10^{11} vg (Karumuthil-Melethil et al. 2016). The low dose (2x10^{11}) in PND11 mice by the IT-L route improved the pathology and reduced psychosine levels, but did not restore them to baseline levels.

A long-term follow-up study was conducted separately to evaluate 8x10^{11} vg dose (4X higher than above) of AAV9/mGALC in twitcher mice by IT-L administration. Seven treated twitcher mice were followed in this study, only weight and survival were assessed in these mice Death in these cohorts was a predefined humane endpoint set to a loss in 20%

body weight. Compared to the cohort at $2x10^{11}$ vg, the treated mice had an extended median lifespan (40 days for untreated, 79 days at $2x10^{11}$ vg, and 140 days at $8x10^{11}$ vg). The improved survival of the mice receiving $8x10^{11}$ vg was statistically significant compared to untreated mice or mice receiving $2x10^{11}$ vg ($P<0.0001$ for each, Mantel-Cox Logrank). The high dose of $8x10^{11}$ correlates to a dose of approximately $2.7x10^{12}$ vg per gram brain weight, which would extrapolate to a dose of $7.7x10^{13}$ vg in a PND14 Krabbe dog and $1.1x10^{15}$ vg in a newborn human.

EXAMPLE 4: GALC delivery in Krabbe dogs improved survival and clinical disease.

Krabbe/GLD in dogs is a relevant model for evaluation of safety, efficacy and is amenable to the clinical route of AAV9/GALC administration. Krabbe is hereditary in dogs and the disease progression closely recapitulates human manifestation (Victoria et al. 1996 Genomics 33(3):457-462). Dogs are the only naturally occurring disease model that results from a missense mutation in the *GALC* gene, (c.473A>C, p.158Y>S), which is inherited as an autosomal recessive trait. Transient transfection of COS-1 cells with the mutant canine GALC cDNA results in no GALC activity in transfected cells, indicating that mutant protein is not functional (Victoria et al 1996). Signs in impacted dogs begin at 4 weeks of age with pelvic limb weakness, thoracic limb dysmetria, and head tremor. Disease then progresses to include ataxia, pelvic limb paresis, urinary incontinence, and loss of hearing by 12 weeks of age. Pelvic limb paralysis warrants euthanasia at 16 weeks (Bradbury et al. 2016 J. Neurosci. Res. 94(11):1007-1017).

In dogs with Krabbe, demyelination reduces motor and sensory conduction velocities in peripheral nerves and can be assayed via nerve conduction studies (Bradbury et al. 2016 J. Neurosci. Res.; McGowan et al. 2000 J. Comput. Assist. Tomogr. 24(2):316-321). Brain stem auditory evoked response (BAER) measurements show increased conduction time. Magnetic resonance imaging (MRI) of the brain shows T2-weighted bilaterally symmetrical increases in signal intensity of the corona radiata, corpus callosum, centrum semiovale, internal capsule and cerebellar white matter compared to a normal, age-matched control dogs. Cerebral ventricles are dilated and sulci are widened indicating cerebral atrophy in Krabbe dogs. Magnetic resonance spectroscopy (MRS) reveals a decrease in N-acetylaspartate indicative of neuronal loss, and increases in choline indicative of abnormal myelin turnover (Vite and Cross 2011 Vet. Radiol. Ultrasound. 52(1 Suppl1):S23-31). Diffusion tensor imaging (DTI) of the canine Krabbe brain shows substantial decreases in fractional anisotropy, increases in

radial diffusivity, and increases in apparent diffusion coefficient in the internal capsule, corona radiata, and corpus callosum when compared to normal dog (Bradbury et al. 2016 Neuroradiol. J. 29(6):417-424; Li et al. 2018 Neuroradiol. J. 31(2):168-176)). Consistent with imaging findings, histologic evaluation shows severe loss of myelin, globoid cell accumulation, and neuroinflammation in the white matter (Bradbury et al. 2016 J. Neurosci. Res.; Bradbury et al. 2018 Hum. Gene Ther. 29(7):785-801).

Brain biochemistry reveals decreased GALC activity and elevated psychosine levels (Bradbury et al. 2016 J. Neurosci. Res.; Wenger et al. 1999 J. Hered. 90(1):138-142). Psychosine is significantly elevated in the serum and CSF at 2 and 4 weeks of age, respectively, and increases steadily over their lifetime in Krabbe dogs. Importantly, psychosine concentration strongly correlates with disease severity. Galactosylceramide, glucosylceramide, and lactosylceramide are also found to be elevated in the CSF of Krabbe dogs and increase with age. The combination of longitudinal electrodiagnostic (nerve conduction velocity and BAER), neuroimaging, and biochemical markers of disease now serve as strong predictive outcome measures in preclinical studies utilizing the canine Krabbe model.

In this study, presymptomatic and symptomatic dogs were administered a single dose of AAV9/cGALC into intrathecal CSF via the cisterna magna injection. Canine Krabbe is the closest in pathology, clinical presentation and progression to replicating Krabbe in humans. The dogs received an immunosuppression regimen, being administered with oral prednisolone for 4 months with a 2 week taper. The study cohort assignment, numbers, age and dose levels tested are listed in **Table 4**.

Table 4: *Treatment cohorts in Krabbe dogs*

GALC genotype	Dosing age (weeks)	Dosing route	No. of Dogs		Dose ($x10^{14}$ vg)	
			Male	Female	Preclinical	Volume (mL)
-/-	2	-	2	2	-	-
-/-	2	IT-CM	4*	6*	1	1
-/-	2	IT-CM	3	1	0.2	1
-/-	6	IT-CM	2	2	1	1
-/-	6	IT-CM	2	2	0.2	1
-/-	3	IT-L	2	2	1	1

All dogs received oral prednisolone for immunosuppression.

Dogs received AAV9/cGALC at two dose levels (**Table 4**). For an interim analysis, 4 treated dogs were euthanized at 16 weeks of age (untreated dog lifespan), to evaluate histopathology, enzyme activity and psychosine accumulation. The remaining dogs will be monitored for the therapeutic efficacy and safety long-term.

The Krabbe dogs receiving immunosuppression regimen alone had a disease course indistinguishable from untreated dogs. They reached a humane endpoint at 11 to 16 weeks of age. Krabbe dogs that received the high dose at 2 weeks of age had no clinical evidence of disease at 16 weeks of age based on physical and neurological evaluation. The rest of the dogs being followed long-term in this cohort are currently 13 - 57 weeks of age with no clinical evidence of disease. Of the dogs that received a lower dose at 2 weeks, 2 of the 3 that were dosed are showing signs of mild to moderate ataxia. Of the dogs that were administered a higher dose at 6 weeks age following the onset of symptoms, 2 of the 3 are showing signs of mild to moderate pelvic limb ataxia.

Clinical severity of the disease correlates well with the severity of demyelination in Krabbe and impacts the nerve conduction velocity (NCV) in humans and animals (Siddiqi et al. 2006 Neurology 67:263-267; Weimer et al. 2005 Muscle & Nerve 32:185-190). IT-CM delivery of AAV9/cGALC resulted in improved and sustained peripheral and sensory NCV. Peripheral NCV testing was performed on untreated Krabbe dogs at humane endpoint (14-19 weeks of age, n=6), normal age-matched control dogs (14-21 weeks, n=9), and AAV9-treated Krabbe dogs every 8 weeks. Motor NCVs were significantly decreased in untreated Krabbe dogs, indicative of demyelination, when compared to age-matched normal control dogs as measured in tibial ($p=0.0093$), sciatic ($p=0.0021$) and ulnar nerves ($p=0.0134$). Sensory NCV of the superficial radial nerve was also significantly reduced ($p=0.0033$) in untreated Krabbe dogs when compared to normal control dogs. Treated dogs improved their NCVs following AAV9/cGALC therapy treading toward the values seen in age matched controls and in some cases performing better (**FIGS. 3A-3C**). These findings suggest gene therapy improved myelination and thus the function in these nerves.

Protein concentration in the CSF is elevated in infants with Krabbe disease within the first weeks of life. A similar increase in CSF protein is seen with disease progression in untreated Krabbe dogs compared to normal age-matched control dogs. Recent evaluation of Krabbe dog CSF by two dimensional difference gel electrophoresis revealed that the source of elevation is likely due to inflammatory activity in the microglia and/or astrocytes. CNS disease progression in the ongoing IT-CM treated dogs was monitored by measuring CSF protein levels monthly. Compared to untreated Krabbe dogs, IT-CM treatment reduced levels of CSF protein (**FIG. 4**).

Magnetic Resonance Imaging (MRI) analysis in symptomatic infantile Krabbe patients showed abnormalities in T2 signal intensity of the cerebral white matter in

periventricular/centrum semiovale, the dentate, cerebellar white matter, thalamus, and parietal-occipital (Abdelhalim et al. 2014 Pediatric Neurology 50:127-134). In canine models of Krabbe, there are areas of demyelination that are detected by MRI (McGowan et al. 2000). The brain of a Krabbe affected dogs shows T2-weighted bilaterally symmetrical increases in signal intensity of the corona radiata (**FIG. 5 panel D, arrow**), corpus callosum, centrum semiovale, internal capsule (**FIG. 5 panel E, arrow**), and cerebellar white matter (**FIG. 5 panel F, arrow**) when compared to a normal, age-matched control dogs (**FIG. 5 panels A-C).** Cerebral ventricles are dilated (**FIG. 5 panel E, arrow**) and sulci are widened (**FIG. 5 panel D, arrow**) indicating cerebral atrophy in Krabbe dogs. In contrast, 16 weeks after IT-CM delivery of AAV9-cGALC white matter signal remains normal at the corpus callosum and internal capsule, although isointensity is noted at the corona radiata and centrum semiovale. Cerebellar white matter is preserved. Ventricles and sulci remain within normal limits indicating attenuation of brain atrophy. Notably, stabilization of MRI changes is seen at 52 weeks of age (**FIG. 5 panels J-L**).

All references cited herein are incorporated herein by reference in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. Although the invention has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the invention as described and defined in the following claims.

What is claimed is:

1. A polynucleotide comprising a canine or human GALC open reading frame, wherein the GALC open reading frame is codon-optimized for expression in a canine or human cell.

2. The polynucleotide of claim 1, wherein said canine or human GALC open reading frame comprises the nucleotide sequence of SEQ ID NO:1 or a nucleotide sequence having at least about 90% identity thereto.

3. The polynucleotide of claim 1, wherein said canine or human GALC open reading frame comprises the nucleotide sequence of SEQ ID NO:2 or a nucleotide sequence having at least about 90% identity thereto.

4. An expression cassette comprising a polynucleotide comprising a canine or human GALC open reading frame.

5. The expression cassette of claim 4, wherein the polynucleotide is the polynucleotide of any one of claims 1-3.

6. The expression cassette of claim 4 or 5, wherein the canine or human GALC open reading frame is operably linked to a promoter.

7. The expression cassette of claim 6, wherein the promoter is a chicken beta actin promoter.

8. The expression cassette of claim 6, wherein the promoter is a JeT promoter.

9. The expression cassette of any one of claims 4-8, wherein the canine or human GALC open reading frame is operably linked to a polyadenylation signal.

10. The expression cassette of claim 9, wherein the polyadenylation signal is a synthetic polyadenylation signal.

11. The expression cassette of claim 9, wherein the polyadenylation signal is an SV40 polyadenylation signal.

12. The expression cassette of any one of claims 4-11, further comprising at least one adeno-associated virus (AAV) inverted terminal repeat (ITR).

13. The expression cassette of claim 12, wherein the expression cassette comprises two AAV ITRs.

14. The expression cassette of claim 12 or 13, wherein the AAV ITRs are AAV2 ITRs.

15. The expression cassette of any one of claims 4-14, wherein the expression cassette is a self-complementary AAV genome.

16. The expression cassette of any one of claims 4-15, wherein the expression cassette comprises a promoter, the canine or human GALC open reading frame, and a polyadenylation site.

17. The expression cassette of any one of claims 4-16, wherein the expression cassette comprises an AAV ITR, a promoter, the canine or human GALC open reading frame, a polyadenylation site, and an AAV ITR.

18. The expression cassette of any one of claims 4-17, wherein the expression cassette comprises an AAV2 ITR, a CAGGS promoter, a human GALC open reading frame, an SV40 polyadenylation site, and an AAV2 ITR.

19. The expression cassette of any one of claims 4-17, wherein the expression cassette comprises an AAV2 ITR, a CAGGS promoter, a canine GALC open reading frame, an SV40 polyadenylation site, and an AAV2 ITR.

20. The expression cassette of any one of claims 4-17, wherein the expression cassette comprises an AAV2 ITR, a JeT promoter, a human GALC open reading frame, a synthetic polyadenylation site, and an AAV2 ITR.

21. The expression cassette of any one of claims 4-17, wherein the expression cassette comprises an AAV2 ITR, a JeT promoter, a canine GALC open reading frame, a synthetic polyadenylation site, and an AAV2 ITR.

22. The expression cassette of claim 18, comprising the nucleotide sequence of SEQ ID NO:8 or a sequence at least about 90% identical thereto.

23. The expression cassette of claim 19, comprising the nucleotide sequence of SEQ ID NO:9 or a sequence at least about 90% identical thereto.

24. The expression cassette of claim 20, comprising the nucleotide sequence of SEQ ID NO:10 or a sequence at least about 90% identical thereto.

25. The expression cassette of claim 21, comprising the nucleotide sequence of SEQ ID NO:11 or a sequence at least about 90% identical thereto.

26. A vector comprising the polynucleotide of any one of claims 1-3 or the expression cassette of any one of claims 4-25.

27. The vector of claim 26, wherein the vector is a viral vector.

28. The vector of claim 26, wherein the vector is an AAV vector.

29. The vector of claim 26, wherein the AAV vector is an AAV9, AAVrh10, AAVOlig001 vector.

30. A transformed cell comprising the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, and/or the vector of any one of claims 26-29.

31. The transformed cell of claim 30, wherein the polynucleotide, expression cassette, and/or vector is stably incorporated into the cell genome.

32. A transgenic animal comprising the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, the vector of any one of claims 26-29, and/or the transformed cell of claim 30 or 31.

33. A pharmaceutical composition comprising the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, the vector of any one of claims 26-29, and/or the transformed cell of claim 30 or 31 in a pharmaceutically acceptable carrier.

34. A method of expressing a GALC open reading frame in a cell, comprising contacting the cell with the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, and/or the vector of any one of claims 26-29, thereby expressing the GALC open reading frame in the cell.

35. A method of expressing a GALC open reading frame in a subject, comprising delivering to the subject the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, the vector of any one of claims 26-29, and/or the transformed cell of claim 30 or 31, thereby expressing the GALC open reading frame in the subject.

36. A method of treating a disorder associated with aberrant expression of a GALC gene or aberrant activity of a GALC gene product in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, the vector of any one of claims 26-29, and/or the transformed cell of claim 30 or 31, such that the GALC open reading frame is expressed in the subject.

37. The method of claim 36, wherein the disorder associated with expression of the GALC gene is Krabbe disease (*i.e.,* globoid cell leukodystrophy).

38. A method of treating Krabbe disease (*i.e.,* globoid cell leukodystrophy) in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, the vector of any one of claims 26-29, and/or the transformed cell of claim 30 or 31, such that the GALC open reading frame is expressed in the subject.

39. The method of any one of claims 36-38, further comprising:

administering to the subject a bone marrow transplant (BMT) prior to administering the effective amount of the polynucleotide of any one of claims 1-3, the expression cassette of any one of claims 4-25, the vector of any one of claims 26-29, and/or the transformed cell of claim 30 or 31.

40. The method of any one of claims 35-39, wherein the subject is a human.

41. The method of any one of claims 35-39, wherein the subject is a dog.

42. The method of any one of claims 35-41, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered or administered to the nervous system of the subject.

43. The method of claim 42, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered or administered by intrathecal, intracerebral, intracerebroventricular, intranasal, intra-aural, intra-ocular, or peri-ocular delivery, or any combination thereof.

44. The method of 42, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered or administered intravenously.

45. The method of claim 42, wherein the polynucleotide, expression cassette, vector, and/or transformed cell is delivered or administered intrathecally.

FIG. 1

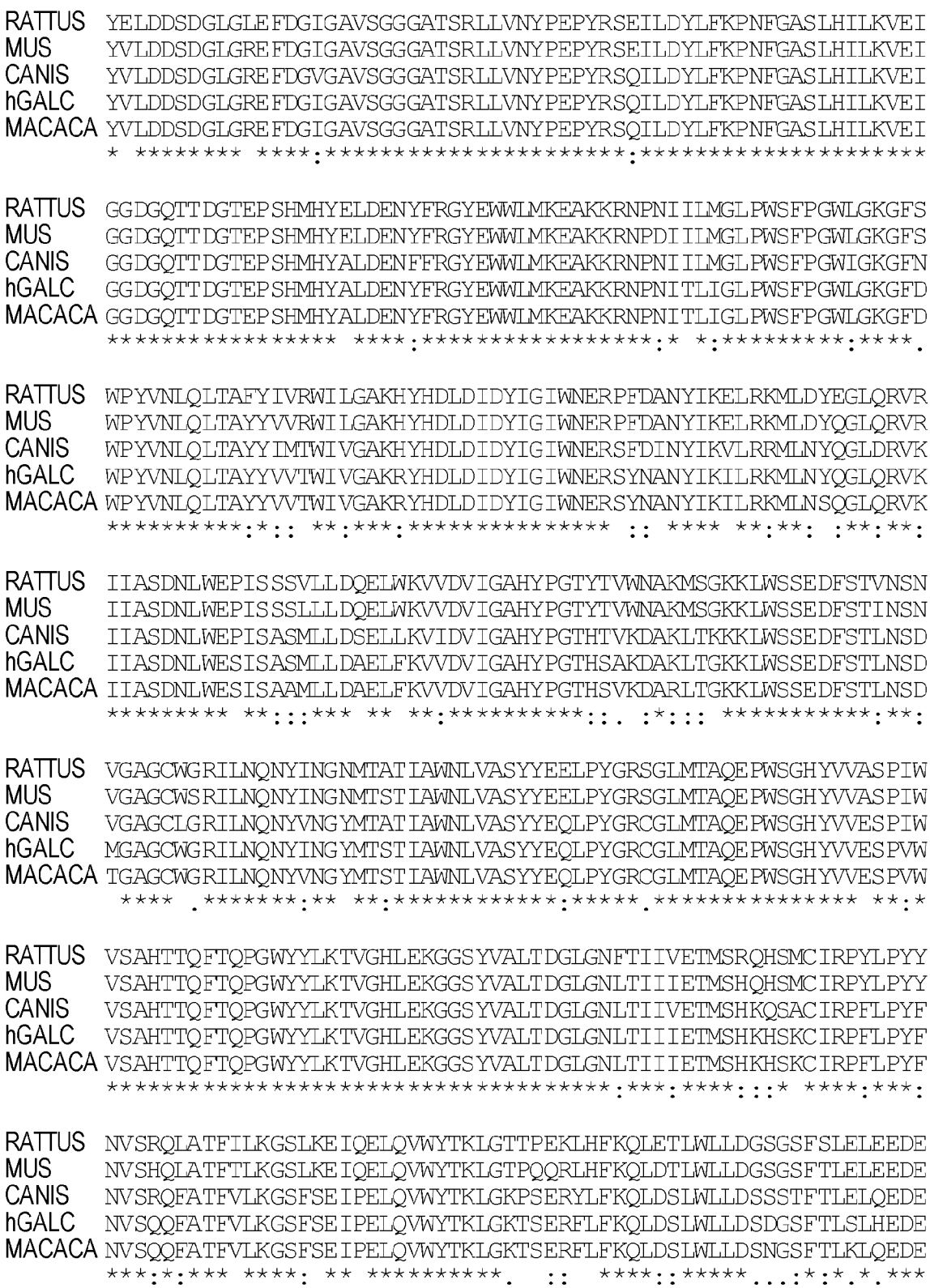

RATTUS YELDDSDGLGLEFDGIGAVSGGGATSRLLVNYPEPYRSEILDYLFKPNFGASLHILKVEI
MUS YVLDDSDGLGREFDGIGAVSGGGATSRLLVNYPEPYRSEILDYLFKPNFGASLHILKVEI
CANIS YVLDDSDGLGREFDGVGAVSGGGATSRLLVNYPEPYRSQILDYLFKPNFGASLHILKVEI
hGALC YVLDDSDGLGREFDGIGAVSGGGATSRLLVNYPEPYRSQILDYLFKPNFGASLHILKVEI
MACACA YVLDDSDGLGREFDGIGAVSGGGATSRLLVNYPEPYRSQILDYLFKPNFGASLHILKVEI
RATTUS GGDGQTTDGTEPSHMHYELDENYFRGYEWWLMKEAKKRNPNIILMGLPWSFPGWLGKGFS
MUS GGDGQTTDGTEPSHMHYELDENYFRGYEWWLMKEAKKRNPDIILMGLPWSFPGWLGKGFS
CANIS GGDGQTTDGTEPSHMHYALDENFFRGYEWWLMKEAKKRNPNIILMGLPWSFPGWIGKGFN
hGALC GGDGQTTDGTEPSHMHYALDENYFRGYEWWLMKEAKKRNPNITLIGLPWSFPGWLGKGFD
MACACA GGDGQTTDGTEPSHMHYALDENYFRGYEWWLMKEAKKRNPNITLIGLPWSFPGWLGKGFD
RATTUS WPYVNLQLTAFYIVRWILGAKHYHDLDIDYIGIWNERPFDANYIKELRKMLDYEGLQRVR
MUS WPYVNLQLTAYYVVRWILGAKHYHDLDIDYIGIWNERPFDANYIKELRKMLDYQGLQRVR
CANIS WPYVNLQLTAYYIMTWIVGAKHYHDLDIDYIGIWNERSFDINYIKVLRRMLNYQGLDRVK
hGALC WPYVNLQLTAYYVVTWIVGAKRYHDLDIDYIGIWNERSYNANYIKILRKMLNYQGLQRVK
MACACA WPYVNLQLTAYYVVTWIVGAKRYHDLDIDYIGIWNERSYNANYIKILRKMLNSQGLQRVK
RATTUS IIASDNLWEPISSSVLLDQELWKVVDVIGAHYPGTYTVWNAKMSGKKLWSSEDFSTVNSN
MUS IIASDNLWEPISSSLLLDQELWKVVDVIGAHYPGTYTVWNAKMSGKKLWSSEDFSTINSN
CANIS IIASDNLWEPISASMLLDSELLKVIDVIGAHYPGTHTVKDAKLTKKKLWSSEDFSTLNSD
hGALC IIASDNLWESISASMLLDAELFKVVDVIGAHYPGTHSAKDAKLTGKKLWSSEDFSTLNSD
MACACA IIASDNLWESISAAMLLDAELFKVVDVIGAHYPGTHSVKDARLTGKKLWSSEDFSTLNSD
RATTUS VGAGCWGRILNQNYINGNMTATIAWNLVASYYEELPYGRSGLMTAQEPWSGHYVVASPIW
MUS VGAGCWSRILNQNYINGNMTSTIAWNLVASYYEELPYGRSGLMTAQEPWSGHYVVASPIW
CANIS VGAGCLGRILNQNYVNGYMTATIAWNLVASYYEQLPYGRCGLMTAQEPWSGHYVVESPIW
hGALC MGAGCWGRILNQNYINGYMTSTIAWNLVASYYEQLPYGRCGLMTAQEPWSGHYVVESPVW
MACACA TGAGCWGRILNQNYVNGYMTSTIAWNLVASYYEQLPYGRCGLMTAQEPWSGHYVVESPVW
RATTUS VSAHTTQFTQPGWYYLKTVGHLEKGGSYVALTDGLGNFTIIVETMSRQHSMCIRPYLPYY
MUS VSAHTTQFTQPGWYYLKTVGHLEKGGSYVALTDGLGNLTIIIETMSHQHSMCIRPYLPYY
CANIS VSAHTTQFTQPGWYYLKTVGHLEKGGSYVALTDGLGNLTIIVETMSHKQSACIRPFLPYF
hGALC VSAHTTQFTQPGWYYLKTVGHLEKGGSYVALTDGLGNLTIIIETMSHKHSKCIRPFLPYF
MACACA VSAHTTQFTQPGWYYLKTVGHLEKGGSYVALTDGLGNLTIIIETMSHKHSKCIRPFLPYF
RATTUS NVSRQLATFILKGSLKEIQELQVWYTKLGTTPEKLHFKQLETLWLLDGSGSFSLELEEDE
MUS NVSHQLATFTLKGSLKEIQELQVWYTKLGTPQQRLHFKQLDTLWLLDGSGSFTLELEEDE
CANIS NVSRQFATFVLKGSFSEIPELQVWYTKLGKPSERYLFKQLDSLWLLDSSSTFTLELQEDE
hGALC NVSQQFATFVLKGSFSEIPELQVWYTKLGKTSERFLFKQLDSLWLLDSDGSFTLSLHEDE
MACACA NVSQQFATFVLKGSFSEIPELQVWYTKLGKTSERFLFKQLDSLWLLDSNGSFTLKLQEDE

FIG. 1 (cont.)

```
RATTUS  MFTLTTLTTGHKGSYRPPPKSQPFPTSYKDDFNVEYPLFSEAPNFADQTGVFEYYTNNED
MUS     IFTLTTLTTGRKGSYPPPPSSKPFPTNYKDDFNVEYPLFSEAPNFADQTGVFEYYMNNED
CANIS   IFTLTTLTVGSKGSYPLPPKSEPFPQIYEDDFDVDYPFFSEAPNFADQTGVFEYFTNIED
hGALC   LFTLTTLTTGRKGSYPLPPKSQPFPSTYKDDFNVDYPFFSEAPNFADQTGVFEYFTNIED
MACACA  LFTLTTLTTGRKGSYLPPPKSQRFPSTYKDDFNVDYPFFSEAPNFADQTGVFEYFTNMED
        :*******.* ****  **.*: **  *:***:*:**:**************** * **

RATTUS  -LEHRFTLRQVLNQRPITWAADASSTISVIGDHHWSNMTVQCDVYIETPRTGGVFIAGRV
MUS     -REHRFTLRQVLNQRPITWAADASSTISVIGDHHWTNMTVQCDVYIETPRSGGVFIAGRV
CANIS   PGEHRFTLRQVLNQRPITWAADAYNTISIIGDYKWSNLTVRCDVYIETPEKGGVFIAGRV
hGALC   PGEHHFTLRQVLNQRPITWAADASNTISIIGDYNWTNLTTKCDVYIETPDTGGVFIAGRV
MACACA  PGEHHFTLRQVLNQRPITWAADASNTISIIGDYNWTNLTIKCDVYIETPDTGGVFIAGRV
          **:****************** .***:***::*:*:* :******** .*********

RATTUS  NKGGILIRTASGVFFWIFANGSYRVTADLGGWITYASGHADVTAKRWYTLTLGIKGYLAS
MUS     NKGGILIRSATGVFFWIFANGSYRVTADLGGWITYASGHADVTAKRWYTLTLGIKGYFAF
CANIS   NKGGILIRSARGIFFWIFANGTYRVTGDLAGWVIYALGRVDVTAKKWYTLTLIIKGRLSS
hGALC   NKGGILIRSARGIFFWIFANGSYRVTGDLAGWIIYALGRVEVTAKKWYTLTLTIKGHFAS
MACACA  NKGGILIRSARGIFFWIFANGSYRVTGDLAGWIIYALGHVEVTAKTWYTLTLTIKGRFAS
        ********:* *:********:****.**.**: ** *:.:**** ****** *** ::

RATTUS  GMLNGKILWENVPVKYPGHGWAAIGTHTFEFAQFDNFHVEAAR 642
MUS     GMLNGTILWKNVRVKYPGHGWAAIGTHTFEFAQFDNFRVEAAR 642
CANIS   GMLNGKTVWKNIPVSFPKNGWAAIGTHSFEFAQFDNFHVEATR 643
hGALC   GMLNDKSLWTDIPVNFPKNGWAAIGTHSFEFAQFDNFLVEATR 643
MACACA  GMLNDKSLWTDIPVNFPKNGWAAIGTHSFEFAQFDNFHVEATR 643
        ****.. :* :: *.:* :********:********* ***:*
```

FIG. 2

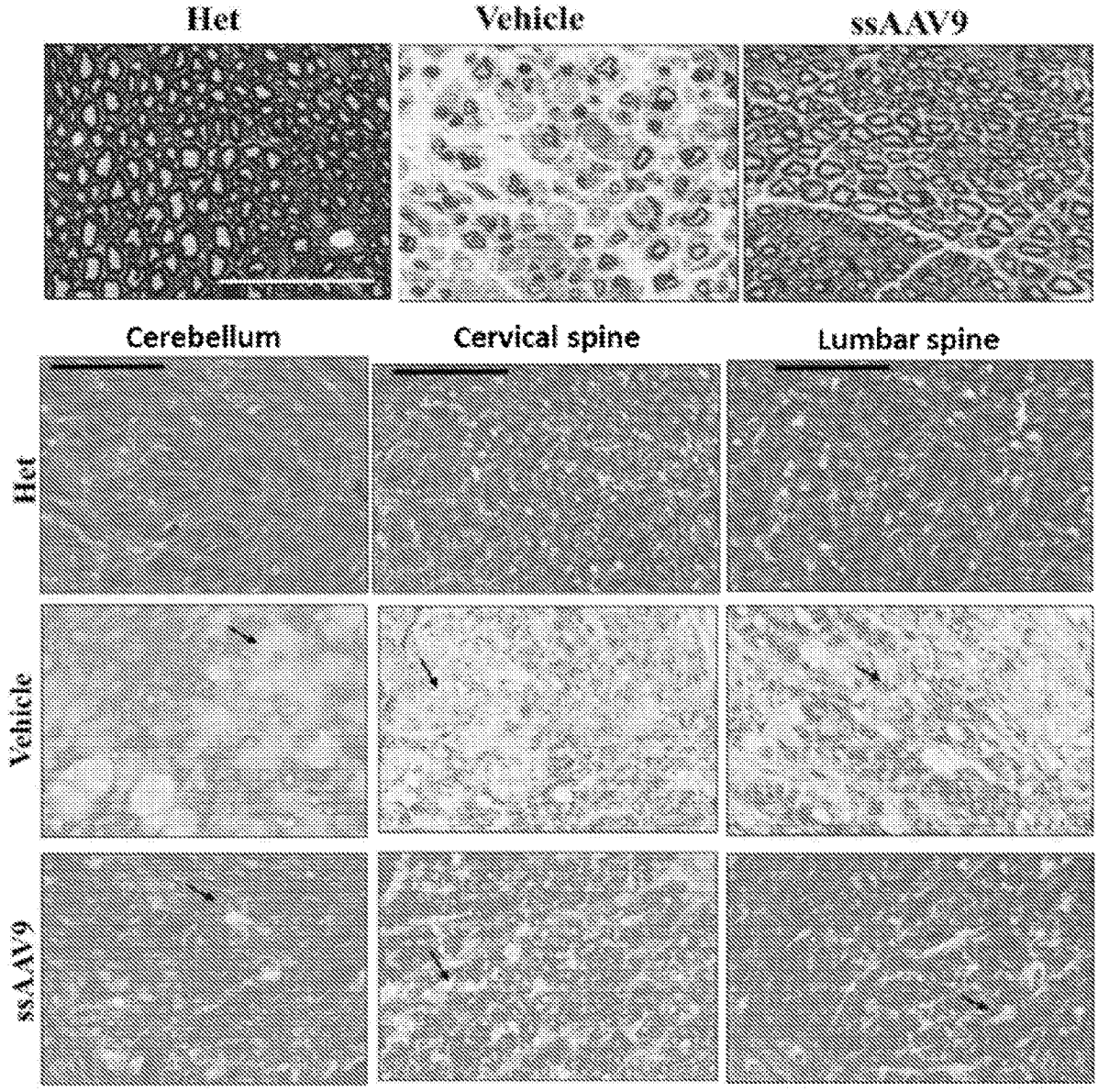

Het
Vehicle
ssAAV9
Cerebellum
Cervical spine
Lumbar spine
Het
Vehicle
ssAAV9

FIG.3A

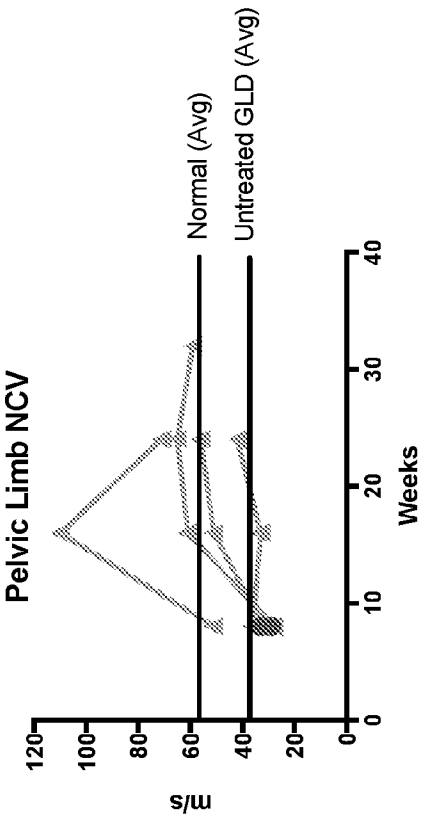


FIG. 3B

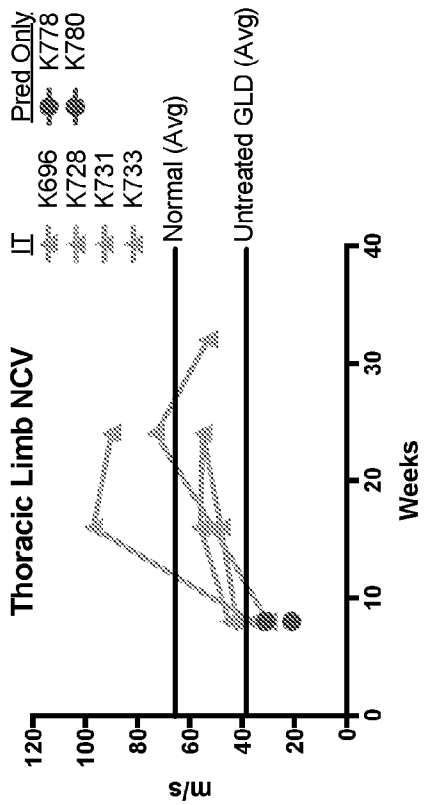


FIG. 3C

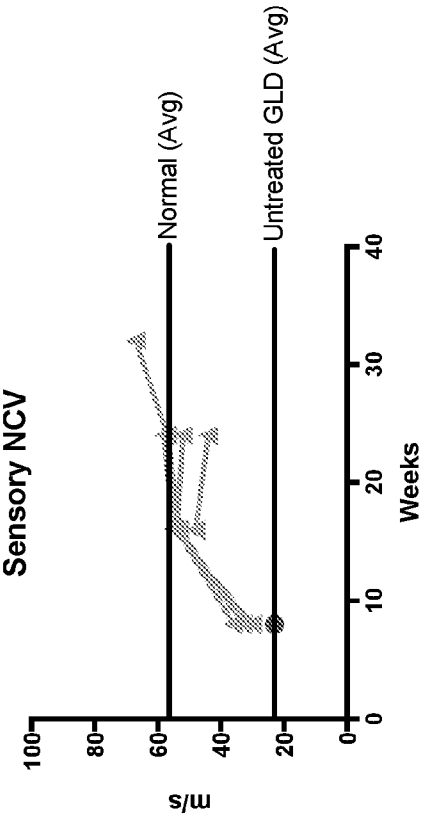


FIG. 4

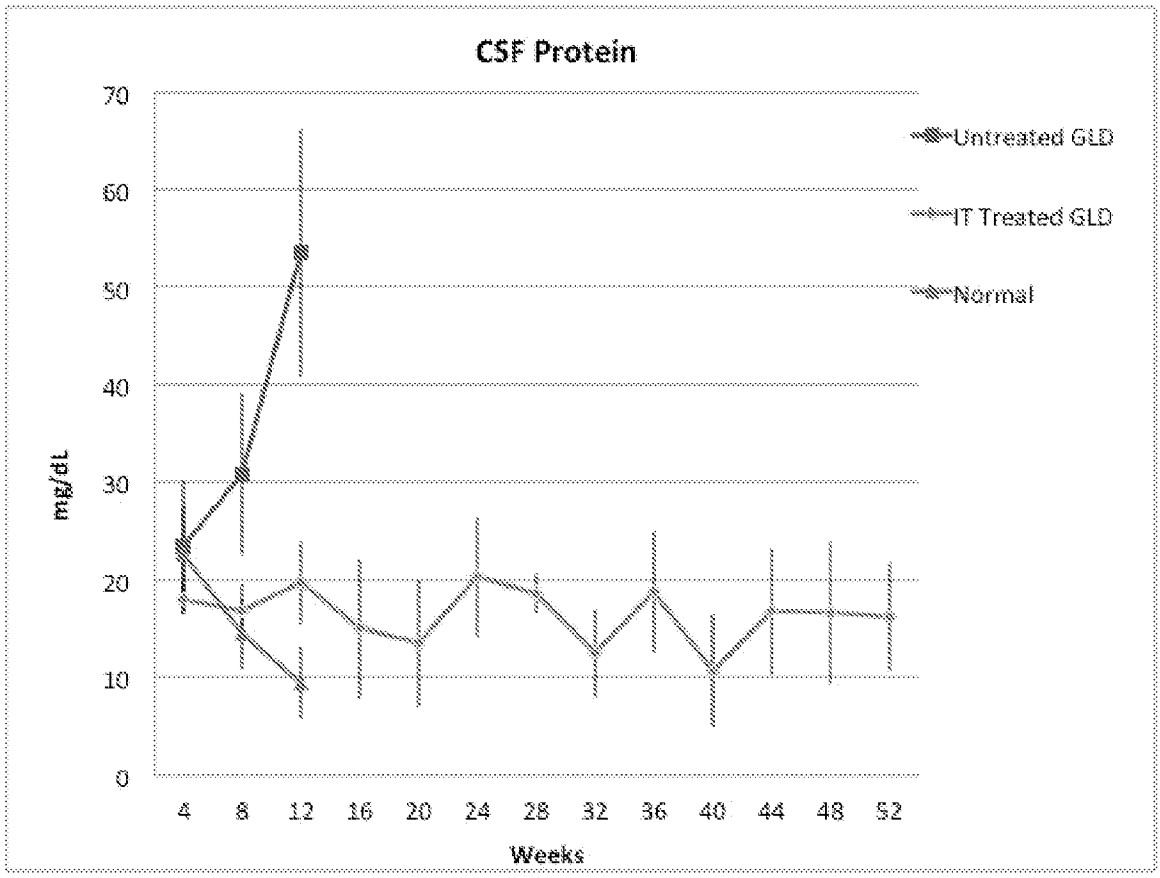
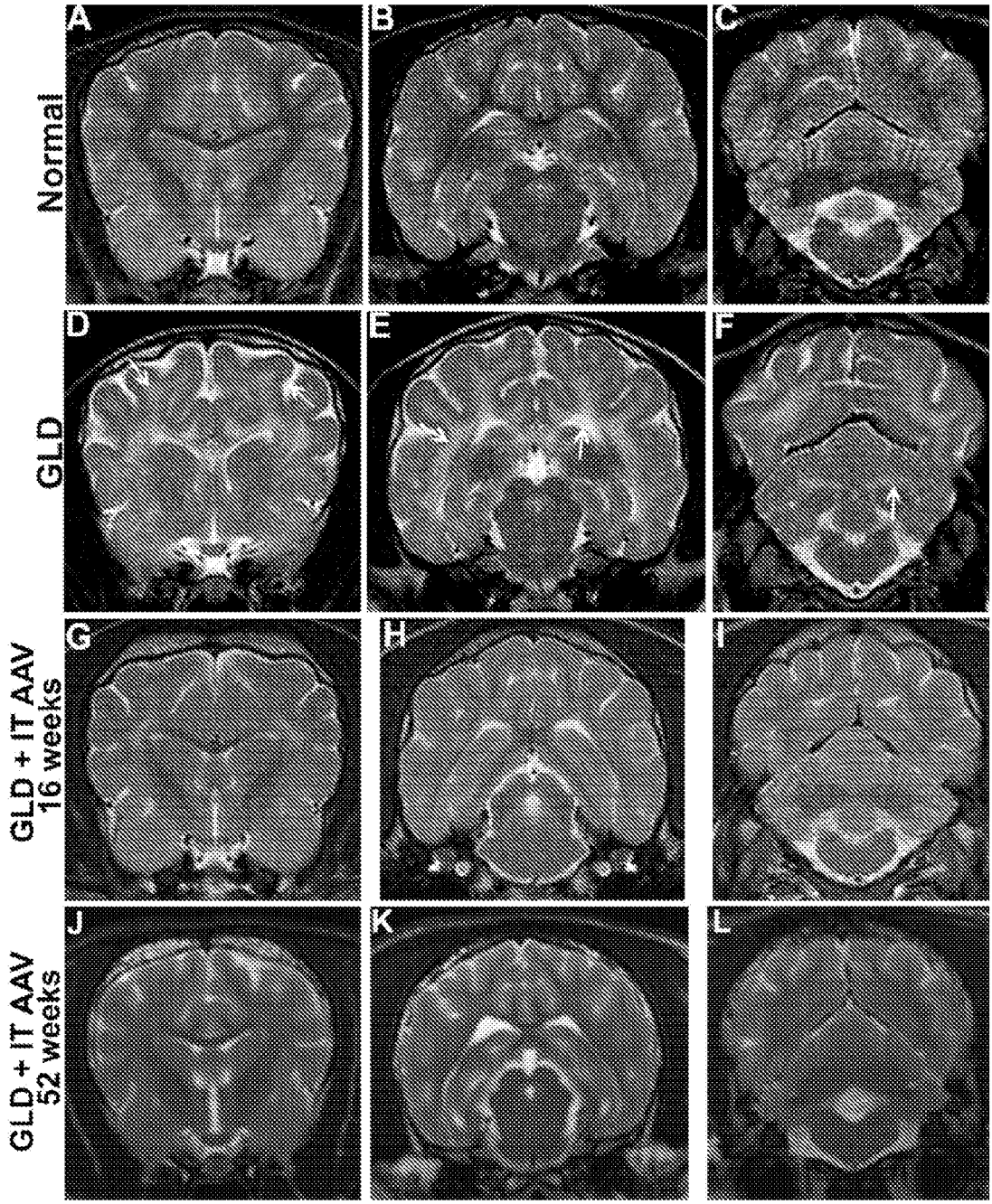

CSF Protein
70
60
50
40
30
20
10
0
mg/dL
4
8
12
16
20
24
28
32
36
40
44
48
52
Weeks
Untreated GLD
IT Treated GLD
Normal

FIG. 5


A
B
C
Normal
D
E
F
GLD
G
H
I
GLD + IT AAV
16 weeks
J
K
L
GLD + IT AAV
52 weeks

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/067727

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.: 34-45
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 34-45 pertain to a method for treatment of the human body by therapy or surgery, 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.: 7-8,10-11,13,22-25,27-29,31,37,43-45
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:
Each of claims 7-8, 10-11, 13, 22-25, 27-29, 31, 37, 43-45 refers to a claim which is not drafted in accordance with Rule 6.4(a).

3. ☒ Claims Nos.: 6,9,12,14-21,26,30,32-36,38-42
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/067727

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/86(2006.01)i, C12N 15/85(2006.01)i, A61K 48/00(2006.01)i, A61P 25/02(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)
C12N 15/86; A61K 48/00; C12N 9/24; C12N 15/85; A61P 25/02

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: GALC, open reading frame, codon-optimization, expression cassette

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN, Y. Q. et al., `Cloning and expression of cDNA encoding human galactocerebrosidase, the enzyme deficient in globoid cell leukodystrophy', Human Molecular Genetics, 1993, vol. 2, no. 11, pp. 1841-1845 abstract; page 1843; figures 1-3	1,4-5
A		2-3
X	VICTORIA, T. et al., `Cloning of the canine GALC cDNA and identification of the mutation causing globoid cell leukodystrophy in West Highland White and Cairn terriers', Genomics, 1996, vol. 33, pp. 457-462 abstract; page 458; figures 1-2	1,4-5
X	UNGARI, S. et al., `Design of a regulated lentiviral vector for hematopoietic stem cell gene therapy of globoid cell leukodystrophy', Mol. Ther. Methods Clin. Dev., 2015, vol. 2, article number:15038, pp. 1-10 abstract; pages 8-9	1,4-5
X	WO 2018-136710 A1 (UNIVERSITY OF PITTSBURGH - OF THE COMMONWEALTH SYSTEM OF HIGHER EDUCATION) 26 July 2018 abstract; pages 23-24; claims 1-18; figure 1	1,4-5
A	US 2014-0221465 A1 (MODERNA THERAPEUTICS, INC.) 07 August 2014 the whole document	1-5

☐ 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
01 May 2020 (01.05.2020)	**01 May 2020 (01.05.2020)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
Facsimile No. +82-42-481-8578

Authorized officer
HEO, Joo Hyung
Telephone No. +82-42-481-8150

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2019/067727

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Information on patent family members

International application No.

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