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An agency of Industry Canada

CA 2973885 A1 2018/01/21

(21) 2 973 885

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(22) Date de dépôt/Filing Date: 2017/07/18

(41) Mise à la disp. pub./Open to Public Insp.: 2018/01/21

(30) Priorité/Priority: 2016/07/21 (US62/365,172)

(51) Cl.Int./Int.Cl. *A61K 31/662* (2006.01),
A61P 3/00 (2006.01)

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(54) Titre : COMPOSITIONS THERAPEUTIQUES RENFERMANT DES COMPOSES DE CREATINE CIBLANT LES MITOCHONDRIES ET LEURS UTILISATIONS EN VUE DE TRAITER ET PREVENIR LES MALADIES ET TROUBLES MITOCHONDRIAUX

(54) Title: THERAPEUTIC COMPOSITIONS INCLUDING MITOCHONDRIAL-TARGETED CREATINE COMPOUNDS AND USES THEREOF TO TREAT AND PREVENT MITOCHONDRIAL DISEASES AND CONDITIONS

(57) Abrégé/Abstract:
Disclosed herein are methods and compositions related to the treatment and/or amelioration of diseases and conditions comprising administration of mitochondrial-targeted creatine compounds, and/or derivatives, analogues, or pharmaceutically acceptable salts thereof. In particular, the present technology relates to administering an effective amount of a mitochondrial-targeted creatine compound to a subject in need thereof to prevent or treat a disease or medical condition, reduce risk factors associated with a disease or medical condition, and/or reducing the severity of a medical disease or condition.

ABSTRACT

Disclosed herein are methods and compositions related to the treatment and/or amelioration of diseases and conditions comprising administration of mitochondrial-targeted creatine compounds, and/or derivatives, analogues, or pharmaceutically acceptable salts thereof. In particular, the present technology relates to administering an effective amount of a mitochondrial-targeted creatine compound to a subject in need thereof to prevent or treat a disease or medical condition, reduce risk factors associated with a disease or medical condition, and/or reducing the severity of a medical disease or condition.

THERAPEUTIC COMPOSITIONS INCLUDING MITOCHONDRIAL-TARGETED CREATINE COMPOUNDS AND USES THEREOF TO TREAT AND PREVENT MITOCHONDRIAL DISEASES AND CONDITIONS

TECHNICAL FIELD

[0001] Disclosed herein are methods and compositions related to the treatment and/or amelioration of diseases and conditions comprising administration of mitochondrial-targeted creatine compounds, and/or derivatives, analogues, or pharmaceutically acceptable salts thereof. In particular, the present technology relates to administering an effective amount of mitochondrial-targeted creatine compounds to a subject in need thereof to prevent or treat a disease or medical condition, reduce risk factors associated with a disease or medical condition, and/or reducing the severity of a medical disease or condition.

BACKGROUND

[0002] The following description is provided to assist the understanding of the reader. None of the information provided or references cited is admitted to be prior art to the present technology.

[0003] Mitochondria are sometimes described as cellular "power plants" because among other things, mitochondria are responsible for creating more than 90% of the energy needed by the body to sustain life and support growth. Mitochondria are organelles found in almost every cell in the body. In addition to making energy, mitochondria are also deeply involved in a variety of other activities, such as making steroid hormones and manufacturing the building blocks of DNA. Mitochondrial failure causes cell injury that leads to cell death.

[0004] Mitochondrial diseases are nearly as common as childhood cancer. Approximately one in 4,000 children born in the United States every year will develop a mitochondrial disorder by age 10. In adults, many diseases of aging have been found to have defects of mitochondrial function. These include, but are not limited to, type 2 diabetes, Parkinson's disease, Alzheimer's disease, and cancer. In addition, select drugs can injure the mitochondria.

[0005] There are multiple forms of mitochondrial disease. Mitochondrial disease can manifest as a chronic, genetic disorder that occurs when the mitochondria of the cell fails to

produce enough energy for cell or organ function. Indeed, for many patients, mitochondrial disease is an inherited condition that runs in families (genetic). Mitochondrial disease is inherited in a number of different ways. There is autosomal inheritance, mtDNA inheritance as well as a combination thereof. For example, mutations of genes encoding Complex I – Complex V can contribute to mitochondrial disease in humans. An uncertain percentage of patients acquire symptoms due to other factors, including mitochondrial toxins.

[0006] Mitochondrial disease presents very differently from individual to individual. There is presently no cure for mitochondrial-based disease. Treatment is generally palliative to improve disease symptoms.

SUMMARY

[0007] In one aspect, the present disclosure provides a method for treating or preventing a mitochondrial disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, in combination with one or more additional therapeutic agents selected from the group consisting of: vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents.

[0008] In some embodiments of the method, the mitochondrial disease or disorder is selected from the group consisting of Alexander disease, Alpers Syndrome, Alpha-ketoglutarate dehydrogenase (AKDGH) deficiency, ALS-FTD, Sideroblastic anemia with spinocerebellar ataxia, Pyridoxine-refractory sideroblastic anemia, GRACILE Syndrome, Björnstad Syndrome, Leigh Syndrome, mitochondrial complex III deficiency nuclear type 1 (MC3DN1), combined oxidative phosphorylation deficiency 18 (COXPD18), Thiamine-responsive megaloblastic anemia syndrome (TRMA), Pearson Syndrome, HAM Syndrome, Ataxia, Cataract, and Diabetes Syndrome, MELAS/MERRF Overlap Syndrome, combined oxidative phosphorylation deficiency-14 (COXPD14), Infantile cerebellar-retinal degeneration (ICRD), Charlevoix-Saguenay spastic ataxia, Primary coenzyme Q10 deficiency-1 (COQ10D1), ataxia oculomotor apraxia type 1 (AOA1), Autosomal recessive spinocerebellar ataxia-9/ coenzyme Q10 deficiency-4 (COQ10D4), Ataxia, Pyramidal Syndrome, and Cytochrome Oxidase Deficiency, Friedreich's ataxia, Infantile onset spinocerebellar ataxia (IOSCA)/ Mitochondrial DNA Depletion Syndrome-7, leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation

(LBSL), Autosomal recessive spastic ataxia-3 (SPAX3), MIRAS, SANDO, mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), spastic ataxia with optic atrophy (SPAX4), progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5), mitochondrial complex III deficiency nuclear type 2 (MC3DN2), episodic encephalopathy due to thiamine pyrophosphokinase deficiency/Thiamine Metabolism Dysfunction Syndrome-5 (THMD5), Spinocerebellar ataxia-28 (SCA28), autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN), Dominant Optic Atrophy (DOA), cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) Syndrome, spinocerebellar ataxia 7 (SCA7), Barth Syndrome, Biotinidase deficiency, gyrate atrophy, Syndromic Dominant Optic Atrophy and Deafness (Syndromic DOAD), Dominant Optic Atrophy plus (DOAplus), Leber's hereditary optic neuropathy (LHON), Wolfram Syndrome-1 (WFS1), Wolfram Syndrome-2 (WFS2), Age-related macular degeneration (ARMD), Brunner Syndrome, Left ventricular noncompaction-1 (LVNC1), histiocytoid cardiomyopathy, Familial Myalgia Syndrome, Parkinsonism, Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency-1 (CEMCOX1), Sengers Syndrome, Cardiofaciocutaneous Syndrome-1 (CFC1), Mitochondrial trifunctional protein (MTP) deficiency, infantile encephalocardiomyopathy with cytochrome c oxidase deficiency, cardiomyopathy + encephalomyopathy, mitochondrial phosphate carrier deficiency, infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency (CEMCOX2), β-Hydroxyisobutyryl CoA Deacylase (HIBCH) deficiency, ECHS1) deficiency, Maternal Inheritance Leigh Syndrome (MILS), dilated cardiomyopathy with ataxia (DCMA), Mitochondrial DNA Depletion Syndrome-12 (MTDPS12), cardiomyopathy due to mitochondrial tRNA deficiencies, mitochondrial complex V (ATP synthase) deficiency nuclear type 1 (MC5DN1), combined oxidative phosphorylation deficiency-8 (COXPD8), progressive leukoencephalopathy with ovarian failure (LKENP), combined oxidative phosphorylation deficiency-10 (COXPD10), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-17 (COXPD17), combined oxidative phosphorylation deficiency-5 (COXPD5), combined oxidative phosphorylation deficiency-9 (COXPD9), carnitine acetyltransferase (CRAT) deficiency, carnitine palmitoyltransferase I (CPT I) deficiency, myopathic carnitine deficiency, primary systemic carnitine deficiency (CDSP), carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acylcarnitine translocase deficiency (CACTD), cartilage-hair hypoplasia, cerebrotendinous xanthomatosis (CTX), congenital adrenal hyperplasia (CAH), megaconial type congenital muscular dystrophy, cerebral creatine deficiency syndrome-3

(CCDS3), maternal nonsyndromic deafness, maternal nonsyndromic deafness, autosomal dominant deafness-64 (DFNA64), Mohr-Tranebjaerg Syndrome, Jensen Syndrome, MEGDEL, reticular dysgenesis, primary coenzyme Q10 deficiency-6 (COQ10D6), CAGSSS, diabetes, Dimethylglycine dehydrogenase deficiency (DMGDHD), Multiple Mitochondrial Dysfunctions Syndrome-1 (MMDS1), Multiple Mitochondrial Dysfunctions Syndrome-2 (MMDS2), Multiple Mitochondrial Dysfunctions Syndrome-3 (MMDS3), childhood leukoencephalopathy associated with mitochondrial Complex II deficiency, encephalopathies associated with mitochondrial Complex I deficiency, encephalopathies associated with mitochondrial Complex III deficiency, encephalopathies associated with mitochondrial Complex IV deficiency, encephalopathies associated with mitochondrial Complex V deficiency, hyperammonemia due to carbonic anhydrase VA deficiency (CA5AD), early infantile epileptic encephalopathy-3 (EIEE3), 2,4-Dienoyl-CoA reductase deficiency (DECRD), infection-induced acute encephalopathy-3 (IIAE3), ethylmalonic encephalopathy (EE), hypomyelinating leukodystrophy (HLD4), exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis, Glutaric aciduria type 1 (GA-1), glycine encephalopathy (GCE), hepatic failure, 2-hydroxyglutaric aciduria, 3-hydroxyacyl-CoA dehydrogenase deficiency, familial hyperinsulinemic hypoglycemia (FHH), hypercalcemia infantile, hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) Syndrome, Immunodeficiency with hyper-IgM type 5 (HIGM5), Inclusion Body Myositis (IBM), polymyositis with mitochondrial pathology, IM-Mito, granulomatous myopathies with anti-mitochondrial antibodies, necrotizing myopathy with pipestem capillaries, myopathy with deficient chondroitin sulfate C in skeletal muscle connective tissue, benign acute childhood myositis, idiopathic orbital myositis, masticator myopathy, hemophagocytic lymphohistiocytosis, infection-associated myositis, Facioscapulohumeral dystrophy (FSH), familial idiopathic inflammatory myopathy, Schmidt Syndrome (Diabetes mellitus, Addison disease, Myxedema), TNF receptor-associated Periodic Syndrome (TRAPS), focal myositis, autoimmune fasciitis, Spanish toxic oil-associated fasciitis, Eosinophilic fasciitis, Macrophagic myofasciitis, Graft-vs-host disease fasciitis, Eosinophilia-myalgia Syndrome, perimyositis, isovaleric acidemia (IVA), Kearnes-Sayre Syndrome (KSS), 2-oxoadipic aciduria, 2-aminoadipic aciduria, Limb-girdle Muscular Dystrophy Syndromes, leukodystrophy, Maple syrup urine disease (MSUD), 3-Methylcrotonyl-CoA carboxylase (MCC), Methylmalonic aciduria (MMA), Miller Syndrome, Mitochondrial DNA Depletion Syndrome-2 (MTDPS2), spinal muscular atrophy syndrome, rigid spine syndrome, severe myopathy with motor regression, Mitochondrial DNA Depletion Syndrome-3, MELAS

Syndrome, camptocormia, MNGIE, MNGIM Syndrome, Menkes Disease, Occipital Horn Syndrome, X-linked distal spinal muscular atrophy-3 (SMAX3), methemoglobinemia, MERRF, progressive external ophthalmoplegia with myoclonus, deafness and diabetes (DD), multiple symmetric lipomatosis, Myopathy with Episodic high Creatine Kinase (MIMECK), Epilepsia Partialis Continua, malignant hyperthermia syndromes, glycogen metabolic disorders, fatty acid oxidation and lipid metabolism disorders, medication-, drug- or toxin-induced myoglobinuria, mitochondrial disorder-associated myoglobinuria, hypokalemic myopathy and rhabdomyolysis, muscle trauma-associated myoglobinuria, ischemia-induced myoglobinuria, infection-induced myoglobinuria, immune myopathies associated with myoglobinuria, Myopathy, lactic acidosis, and sideroblastic anemia (MLASA), infantile mitochondrial myopathy due to reversible COX deficiency (MMIT), Myopathy, Exercise intolerance, Encephalopathy and Lactic acidemia Syndrome, myoglobinuria and exercise intolerance syndrome, exercise intolerance, proximal weakness ± myoglobinuria syndrome, encephalopathy and seizures syndrome, septo-optic dysplasia, exercise intolerance ± mild weakness, myopathy ± exercise intolerance, growth or CNS disorder, maternally-inherited mitochondrial myopathies, myopathy with lactic acidosis, myopathy with rhabdomyolysis, Myopathy with cataract and combined respiratory chain deficiency, myopathy with abnormal mitochondrial translation, Fatigue Syndrome, myopathy with extrapyramidal movement disorders (MPXPS), glutaric aciduria II (MADD), primary CoQ10 deficiency-1 (COQ10D1), primary CoQ10 deficiency-2 (COQ10D2), primary CoQ10 deficiency-3 (COQ10D3), primary CoQ10 deficiency-5 (COQ10D5), secondary CoQ10 deficiency, autosomal dominant mitochondrial myopathy, myopathy with focal depletion of mitochondria, mitochondrial DNA breakage syndrome (PEO + Myopathy), lipid type mitochondrial myopathy, multiple symmetric lipomatosis (MSL), N-acetylglutamate synthase (NAGS) deficiency, Nephronophthisis (NPHP), ornithine transcarbamylase (OTC) deficiency, neoplasms, NARP Syndrome, paroxysmal nonkinesigenic dyskinesia (PNKD), sporadic PEO, maternally-inherited PEO, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-3 (PEOA3), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-2 (PEOA2), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-1 (PEOA1), PEO+ demyelinating neuropathy, PEO + hypogonadism, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-4 (PEOA4), distal myopathy, cachexia & PEO, autosomal dominant progressive external ophthalmoplegia-6 (PEOA6), PEO + Myopathy and Parkinsonism, autosomal recessive progressive external ophthalmoplegia

(PEOB), Mitochondrial DNA Depletion Syndrome-11 (MTDPS11), PEO with cardiomyopathy, PEPCK deficiency, Perrault Syndromes (PRLTS), propionic acidemia (PA), pyruvate carboxylase deficiency, pyruvate dehydrogenase E1-alpha deficiency (PDHAD), pyruvate dehydrogenase E1-beta deficiency (PDHBD), dihydrolipoamide dehydrogenase (DLD) deficiency, pyruvate dehydrogenase phosphatase deficiency, pyruvate dehydrogenase E3-binding protein deficiency (PDHXD), mitochondrial pyruvate carrier deficiency (MPYCD), Schwartz-Jampel Syndrome type 1 (SJS1), selenium deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency, succinyl CoA:3-oxacid CoA transferase (SCOT) deficiency, Stuve-Wiedemann Syndrome (STWS), thrombocytopenia (THC), Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), Vitamin D-dependent rickets type 1A (VDDR1A), Wilson's disease, Zellweger Syndrome (PBD3A), arsenic trioxide myopathy, myopathy and neuropathy resulting from nucleoside analogues, germanium myopathy, Parkinsonism and mitochondrial Complex I neurotoxicity due to trichloroethylene, valproate-induced hepatic failure, neurodegeneration with brain iron accumulation-4 (NBIA4), Complex I deficiency, Complex II deficiency, Complex III deficiency, Complex IV deficiency, Complex V deficiency, Cytochrome c oxidase (COX) deficiency, combined complex I, II, IV, ± V deficiency, combined complex I, II, and III deficiency, combined oxidative phosphorylation deficiency-1 (COXPD1), combined oxidative phosphorylation deficiency-2 (COXPD2), combined oxidative phosphorylation deficiency-3 (COXPD3), combined oxidative phosphorylation deficiency-4 (COXPD4), combined oxidative phosphorylation deficiency-6 (COXPD6), combined oxidative phosphorylation deficiency-7 (COXPD7), combined oxidative phosphorylation deficiency-9 (COXPD9), combined oxidative phosphorylation deficiency-11 (COXPD11), combined oxidative phosphorylation deficiency-12 (COXPD12), combined oxidative phosphorylation deficiency-13 (COXPD13), combined oxidative phosphorylation deficiency-15 (COXPD15), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-19 (COXPD19), combined oxidative phosphorylation deficiency-20 (COXPD20), combined oxidative phosphorylation deficiency-21 (COXPD21), fumarase deficiency, HMG-CoA synthase-2 deficiency, hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (HUPRA) Syndrome, syndromic microphthalmia-7, pontocerebellar hypoplasia type 6 (PCH6), Mitochondrial DNA Depletion Syndrome-9 (MTDPS9P), and Sudden infant death Syndrome (SIDS).

[0009] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered one, two, three, four, or five times per day. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered more than five times per day.

[0010] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered every day, every other day, every third day, every fourth day, every fifth day, or every sixth day. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered weekly, bi-weekly, tri-weekly, or monthly.

[0011] In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of one, two, three, four, or five weeks. In some embodiments, the mitochondrial-targeted creatine compound is administered for six weeks or more. In some embodiments, the mitochondrial-targeted creatine compound is administered for twelve weeks or more. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of less than one year. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of more than one year.

[0012] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for one, two, three, four or five weeks. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for less than 6 weeks. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for 6 weeks or more. In other embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for 12 weeks or more.

[0013] In some embodiments of the method, the subject displays abnormal levels of one or more energy biomarkers compared to a normal control subject. In some embodiments, the energy biomarker is selected from the group consisting of lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; lactate/pyruvate ratios; total, reduced or oxidized glutathione levels; reduced/oxidized glutathione ratios; total, reduced or oxidized cysteine levels; reduced/oxidized cysteine ratios; phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized

cytochrome C levels; reduced cytochrome C levels; oxidized cytochrome C/reduced cytochrome C ratio; acetoacetate levels; beta-hydroxy butyrate levels; acetoacetate/ beta-hydroxy butyrate ratio; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; levels of reactive oxygen species; oxygen consumption (VO2), carbon dioxide output (VCO2), and respiratory quotient (VCO2/VO2). In some embodiments of the method, the lactate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the pyruvate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the lactate/pyruvate ratios of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized glutathione levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized cysteine levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the reduced/oxidized glutathione ratios of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the reduced or oxidized cysteine ratios of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject.

[0014] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly. In some embodiments of the method, the subject is human.

[0015] Additionally or alternatively, in some embodiments of the method, the symptoms of the mitochondrial disease or disorder comprises one or more of poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid

dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, and brain atrophy.

[0016] Additionally or alternatively, in some embodiments, the mitochondrial-targeted creatine compound and the one or more additional therapeutic agents are administered separately, sequentially or simultaneously to the subject.

[0017] In another aspect, the present disclosure provides a method for modulating the expression of one or more energy biomarkers in a mammalian subject in need thereof, the method comprising: administering to the subject a therapeutically effective amount of a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, in combination with one or more additional therapeutic agents selected from the group consisting of: vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents.

[0018] In some embodiments of the method, the energy biomarker is selected from the group consisting of lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; lactate/pyruvate ratios; total, reduced or oxidized glutathione levels; reduced/oxidized glutathione ratios; total, reduced or oxidized cysteine levels; reduced/oxidized cysteine ratios; phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; oxidized cytochrome C/reduced cytochrome C ratio; acetoacetate levels; beta-hydroxy butyrate levels; acetoacetate/ beta-hydroxy butyrate ratio; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; levels of reactive oxygen species; oxygen consumption (VO2), carbon dioxide output (VCO2), and respiratory quotient (VCO2/VO2). In some embodiments of the method, the lactate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the pyruvate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the lactate/pyruvate ratios of one or more of whole blood,

plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized glutathione levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized cysteine levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the reduced/oxidized glutathione ratios of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the reduced or oxidized cysteine ratios of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject.

[0019] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered one, two, three, four, or five times per day. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered more than five times per day.

[0020] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered every day, every other day, every third day, every fourth day, every fifth day, or every sixth day. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered weekly, bi-weekly, tri-weekly, or monthly.

[0021] In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of one, two, three, four, or five weeks. In some embodiments, the mitochondrial-targeted creatine compound is administered for six weeks or more. In some embodiments, the mitochondrial-targeted creatine compound is administered for twelve weeks or more. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of less than one year. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of more than one year.

[0022] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for one, two, three, four or five weeks. In some embodiments of the method, the mitochondrial-targeted creatine

compound is administered daily for less than 6 weeks. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for 6 weeks or more. In other embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for 12 weeks or more.

[0023] In some embodiments of the method, the subject has been diagnosed as having, is suspected of having, or is at risk of having a mitochondrial disease or disorder. In a further embodiment of the method, the subject is human.

[0024] In some embodiments of the method, the symptoms of the mitochondrial disease or disorder comprises one or more of poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, and brain atrophy.

[0025] In some embodiments of the method, the mitochondrial-targeted creatine compound is administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly.

[0026] Additionally or alternatively, in some embodiments, the mitochondrial-targeted creatine compound and the one or more additional therapeutic agents are administered separately, sequentially or simultaneously to the subject.

[0027] In another aspect, the present technology provides methods for treating, ameliorating or preventing the disruption of mitochondrial oxidative phosphorylation in a subject in need thereof, by administering mitochondrial-targeted creatine compounds as disclosed herein, the method comprising administering to the subject a therapeutically effective amount of a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, in combination with one or more additional therapeutic agents selected from the group consisting of: vitamins, cofactors, antibiotics, hormones,

antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents, thereby preventing, ameliorating, or treating mitochondrial oxidative phosphorylation, and/or signs or symptoms thereof.

[0028] In some embodiments of the method, the subject is suffering from or is at increased risk of a disruption of mitochondrial oxidative phosphorylation. In some embodiments, the subject is suffering from or is at increased risk of a disease or conditions characterized by a genetic mutation which affects mitochondrial function.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. 1 shows the effect that various dysfunctions can have on energy biomarkers as well as biochemical events that occur within the body. It also indicates the physical effect (such as a disease symptom or other effect of the dysfunction) typically associated with a given dysfunction. It should be noted that any of the energy biomarkers listed in the table, in addition to energy biomarkers enumerated elsewhere, can also be modulated, enhanced, or normalized by the mitochondrial-targeted creatine compounds of the present technology. RQ=respiratory quotient; BMR=basal metabolic rate; HR (CO)=heart rate (cardiac output); T=body temperature (preferably measured as core temperature); AT=anaerobic threshold; pH=blood pH (venous and/or arterial).

DETAILED DESCRIPTION

[0030] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present technology are described below in various levels of detail in order to provide a substantial understanding of the present technology. The definitions of certain terms as used in this specification are provided below. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which the present technology belongs.

[0031] All numerical designations, *e.g.*, pH, temperature, time, concentration and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate, or alternatively by a variation of +/- 10%, or alternatively 5% or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about".

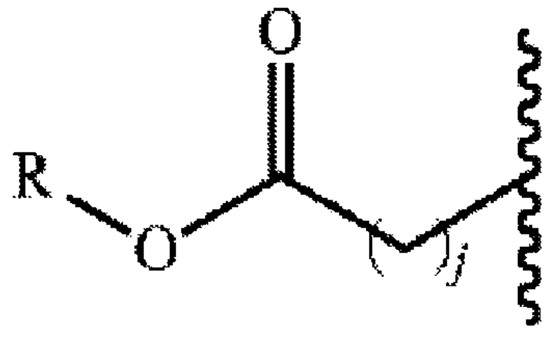
[0032] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a cell" includes a combination of two or more cells, and the like.

[0033] As used herein, the "administration" of an agent, drug, or compound to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including orally, intranasally, intrathecally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), intraocularly, intradermally, transmucosally, iontophoretically, or topically. Administration includes self-administration and the administration by another.

[0034] The term "alkyl", with respect to the mitochondrial-targeted creatine compounds disclosed herein, refers to the radical of saturated or unsaturated aliphatic groups, including straight-chain alkyl, alkenyl, or alkynyl groups, branched-chain alkyl, alkenyl, or alkynyl groups, cycloalkyl, cycloalkenyl, or cycloalkynyl (alicyclic) groups, alkyl substituted cycloalkyl, cycloalkenyl, or cycloalkynyl groups, and cycloalkyl substituted alkyl, alkenyl, or alkynyl groups. Unless otherwise indicated, a straight chain or branched chain alkyl has 30 or fewer carbon atoms, or 20 or fewer carbon atoms in its backbone (*e.g.*, C_1-C_{30} for straight chain, C_3-C_{30} for branched chain). Likewise, some cycloalkyls have from 3-10 carbon atoms in their ring structure, or have 5, 6 or 7 carbons in the ring structure. The alkyl groups can also be substituted with one or more groups including, but not limited to, halogen, hydroxy, amino, thio, ether, ester, carboxy, oxo, and aldehyde groups. The alkyl groups may also contain one or more heteroatoms.

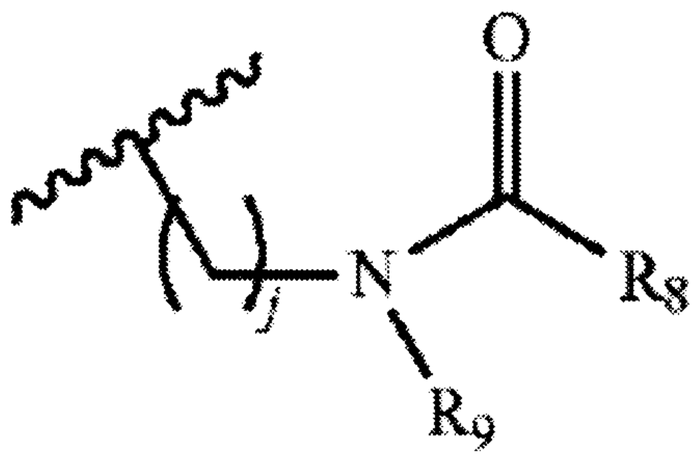
[0035] The terms "alkenyl" and "alkynyl" refer specifically to unsaturated aliphatic groups containing one or more double or triple bonds analogous in length (*e.g.*, C_2-C_{30}) and possible substitution to the alkyl groups described above.

[0036] "Alkoxycarbonyl", as used herein, refers to a substituent having the following chemical formula:



wherein R is a linear, branched, or cyclic alkyl group, wherein j is from about 1 to about 12.

[0037] "Alkoxycarbamido", as used herein, refers to a substituent having the following chemical formula:



wherein R_8 is alkoxy and R_9 is hydrogen, alkoxy-alkyl, or alkanoyl, and j is from about 1 to about 12.

[0038] "Alkylaryl", as used herein, refers to an alkyl group substituted with an aryl group (*e.g.*, an aromatic or hetero aromatic group).

[0039] As used herein, the term "amino acid" includes naturally-occurring amino acids and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally-occurring amino acids. Naturally-occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally-occurring amino acid, *i.e.*, an α-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally-occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally-occurring amino acid. Amino acids can be referred to herein by either their commonly

known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0040] "Aryl", with respect to the mitochondrial-targeted creatine compounds disclosed herein, refers to 5-, 6-, and 7-membered aromatic, heterocyclic, fused aromatic, fused heterocyclic, biaromatic, or biheterocyclic ring system, optionally substituted by halogens, alkyl-, alkenyl-, and alkynyl-groups. Broadly defined, "Ar", includes 5-6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, —CF_3, —CN, or the like. The term "Ar" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles. Examples of heterocyclic rings include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H, 6H-1,5,2-dithiazinyl, dihydrofuro[2,3b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl,

quinazolinyl, quinolinyl, 4H-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl.

[0041] The term "chiral center" refers to a carbon atom to which four different groups are attached. Choice of the appropriate chiral column, eluent, and conditions necessary to effect separation of the pair of enantiomers is well known to one of ordinary skill in the art using standard techniques (see *e.g.* Jacques, J. *et al*, "Enantiomers, Racemates, and Resolutions", John Wiley and Sons, Inc. 1981).

[0042] "Cleavable linker", as used herein, refers to a molecule, moiety, atom, or group of atoms capable of covalently connecting creatine or a creatine analog to a mitochondrial targeting moiety as defined herein.

[0043] As used herein, a "control" is an alternative sample used in an experiment for comparison purpose. A control can be "positive" or "negative." For example, where the purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

[0044] As used herein the term "diastereomer" refers to two stereoisomers which are not mirror images but also not superimposable.

[0045] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or amelioration of a disease or medical condition described herein or one or more symptoms associated with a disease or medical condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will depend on the type and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. It will also depend on the degree, severity and type of disease. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In some embodiments, an effective amount of a compound is an amount of the compound

sufficient to modulate, normalize, or enhance one or more energy biomarkers (where modulation, normalization, and enhancement are defined herein). In the methods described herein, the compositions of the present technology may be administered to a subject having one or more signs or symptoms of a disease or medical condition described herein. For example, a "therapeutically effective amount" of the mitochondrial-targeted creatine compounds is meant levels in which the physiological effects of a particular disease or medical condition are, at a minimum, ameliorated. A therapeutically effective amount can be given in one or more administrations. By way of example only, in some embodiments, the disease or medical condition is a mitochondrial disease or disorder. In some embodiments, signs, symptoms or complications of a mitochondrial disease or disorder include, but are not limited to: poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, brain atrophy, or any other sign or symptom of a mitochondrial disease state disclosed herein. In other embodiments of the method, the disease or medical condition is selected from the group consisting of vitiligo, porphyria, Alport Syndrome, and IPF.

[0046] As used herein, the term "enantiomers" refers to two stereoisomers which are non-superimposable mirror images of one another.

[0047] As used herein, the terms "enhancement" of, or to "enhance," energy biomarkers means to improve the level of one or more energy biomarkers in a direction that results in a beneficial or desired physiological outcome in a subject (compared to the values observed in a normal control subject, or the value in the subject prior to treatment with a composition or compound). For example, in a situation where significant energy demands are placed on a subject, it may be desirable to increase the level of ATP in that subject to a level above the ATP level observed in a normal control subject. Enhancement can also be of beneficial effect in a subject suffering from a disease or pathology such as a mitochondrial disease, in that normalizing an energy biomarker may not achieve the optimum outcome for the subject; in

such cases, enhancement of one or more energy biomarkers can be beneficial, for example, higher-than-normal levels of ATP, or lower-than normal levels of lactic acid (lactate) can be beneficial to such a subject.

[0048] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in an eukaryotic cell. The expression level of a gene may be determined by measuring the amount of mRNA or protein in a cell or tissue sample. In one aspect, the expression level of a gene from one sample may be directly compared to the expression level of that gene from a control or reference sample. In another aspect, the expression level of a gene from one sample may be directly compared to the expression level of that gene from the same sample following administration of a mitochondrial-targeted creatine compound.

[0049] "Halogen", as used herein, refers to fluorine, chlorine, bromine, or iodine.

[0050] "Heteroaryl", as used herein, refers to a monocyclic aromatic ring containing five or six ring atoms consisting of carbon and 1, 2, 3, or 4 heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(Y) where Y is absent or is H, O, (C_1-C_8)alkyl, phenyl or benzyl. Non-limiting examples of heteroaryl groups include furyl, imidazolyl, triazolyl, triazinyl, oxazoyl, isoxazoyl, thiazolyl, isothiazoyl, pyrazolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl, (or its N-oxide), thienyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide), quinolyl (or its N-oxide) and the like. The term "heteroaryl" can include radicals of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto. Examples of heteroaryl can be furyl, imidazolyl, triazolyl, triazinyl, oxazoyl, isoxazoyl, thiazolyl, isothiazoyl, pyraxolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl (or its N-oxide), thientyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide), quinolyl (or its N-oxide), and the like.

[0051] "Heterocycle" or "heterocyclic", as used herein, refers to a cyclic radical attached via a ring carbon or nitrogen of a monocyclic or bicyclic ring containing 3-10 ring atoms, or from 5-6 ring atoms, consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(Y) wherein Y is absent or is H, O,

(C_{1-4})alkyl, phenyl or benzyl, and optionally containing 1-3 double bonds and optionally substituted with one or more substituents. Examples of heterocyclic ring include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolinyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl.

[0052] As used herein, the term "mitochondrial disease or disorder" refers to any disease or disorder that results from the perturbation of any biological/physiological process in the mitochondria. Non-limiting examples of mitochondrial disease include but are not limited to Alexander disease, Alpers Syndrome, Alpha-ketoglutarate dehydrogenase (AKDGH) deficiency, ALS-FTD, Sideroblastic anemia with spinocerebellar ataxia, Pyridoxine-refractory sideroblastic anemia, GRACILE Syndrome, Björnstad Syndrome, Leigh Syndrome, mitochondrial complex III deficiency nuclear type 1 (MC3DN1), combined oxidative phosphorylation deficiency 18 (COXPD18), Thiamine-responsive megaloblastic anemia syndrome (TRMA), Pearson Syndrome, HAM Syndrome, Ataxia, Cataract, and Diabetes Syndrome, MELAS/MERRF Overlap Syndrome, combined oxidative phosphorylation deficiency-14 (COXPD14), Infantile cerebellar-retinal degeneration (ICRD), Charlevoix-Saguenay spastic ataxia, Primary coenzyme Q10 deficiency-1 (COQ10D1), ataxia oculomotor apraxia type 1 (AOA1), Autosomal recessive spinocerebellar ataxia-9/

coenzyme Q10 deficiency-4 (COQ10D4), Ataxia, Pyramidal Syndrome, and Cytochrome Oxidase Deficiency, Friedreich's ataxia, Infantile onset spinocerebellar ataxia (IOSCA)/ Mitochondrial DNA Depletion Syndrome-7, Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), Autosomal recessive spastic ataxia-3 (SPAX3), MIRAS, SANDO, mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), spastic ataxia with optic atrophy (SPAX4), progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5), mitochondrial complex III deficiency nuclear type 2 (MC3DN2), episodic encephalopathy due to thiamine pyrophosphokinase deficiency/Thiamine Metabolism Dysfunction Syndrome-5 (THMD5), Spinocerebellar ataxia-28 (SCA28), autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN), Dominant Optic Atrophy (DOA), cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) Syndrome, spinocerebellar ataxia 7 (SCA7), Barth Syndrome, Biotinidase deficiency, gyrate atrophy, Syndromic Dominant Optic Atrophy and Deafness (Syndromic DOAD), Dominant Optic Atrophy plus (DOAplus), Leber's hereditary optic neuropathy (LHON), Wolfram Syndrome-1 (WFS1), Wolfram Syndrome-2 (WFS2), Age-related macular degeneration (ARMD), Brunner Syndrome, Left ventricular noncompaction-1 (LVNC1), histiocytoid cardiomyopathy, Familial Myalgia Syndrome, Parkinsonism, Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency-1 (CEMCOX1), Sengers Syndrome, Cardiofaciocutaneous Syndrome-1 (CFC1), Mitochondrial trifunctional protein (MTP) deficiency, infantile encephalocardiomyopathy with cytochrome c oxidase deficiency, cardiomyopathy + encephalomyopathy, mitochondrial phosphate carrier deficiency, infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency (CEMCOX2), β-Hydroxyisobutyryl CoA Deacylase (HIBCH) deficiency, ECHS1) deficiency, Maternal Inheritance Leigh Syndrome (MILS), dilated cardiomyopathy with ataxia (DCMA), Mitochondrial DNA Depletion Syndrome-12 (MTDPS12), cardiomyopathy due to mitochondrial tRNA deficiencies, mitochondrial complex V (ATP synthase) deficiency nuclear type 1 (MC5DN1), combined oxidative phosphorylation deficiency-8 (COXPD8), progressive leukoencephalopathy with ovarian failure (LKENP), combined oxidative phosphorylation deficiency-10 (COXPD10), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-17 (COXPD17), combined oxidative phosphorylation deficiency-5 (COXPD5), combined oxidative phosphorylation deficiency-9 (COXPD9), carnitine acetyltransferase (CRAT) deficiency, carnitine palmitoyltransferase I (CPT I) deficiency, myopathic carnitine deficiency, primary systemic

carnitine deficiency (CDSP), carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acylcarnitine translocase deficiency (CACTD), cartilage-hair hypoplasia, cerebrotendinous xanthomatosis (CTX), congenital adrenal hyperplasia (CAH), megaconial type congenital muscular dystrophy, cerebral creatine deficiency syndrome-3 (CCDS3), maternal nonsyndromic deafness, maternal nonsyndromic deafness, autosomal dominant deafness-64 (DFNA64), Mohr-Tranebjaerg Syndrome, Jensen Syndrome, MEGDEL, reticular dysgenesis, primary coenzyme Q10 deficiency-6 (COQ10D6), CAGSSS, diabetes, Dimethylglycine dehydrogenase deficiency (DMGDHD), Multiple Mitochondrial Dysfunctions Syndrome-1 (MMDS1), Multiple Mitochondrial Dysfunctions Syndrome-2 (MMDS2), Multiple Mitochondrial Dysfunctions Syndrome-3 (MMDS3), childhood leukoencephalopathy associated with mitochondrial Complex II deficiency, encephalopathies associated with mitochondrial Complex I deficiency, encephalopathies associated with mitochondrial Complex III deficiency, encephalopathies associated with mitochondrial Complex IV deficiency, encephalopathies associated with mitochondrial Complex V deficiency, hyperammonemia due to carbonic anhydrase VA deficiency (CA5AD), early infantile epileptic encephalopathy-3 (EIEE3), 2,4-Dienoyl-CoA reductase deficiency (DECRD), infection-induced acute encephalopathy-3 (IIAE3), ethylmalonic encephalopathy (EE), hypomyelinating leukodystrophy (HLD4), exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis, Glutaric aciduria type 1 (GA-1), glycine encephalopathy (GCE), hepatic failure, 2-hydroxyglutaric aciduria, 3-hydroxyacyl-CoA dehydrogenase deficiency, familial hyperinsulinemic hypoglycemia (FHH), hypercalcemia infantile, hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) Syndrome, Immunodeficiency with hyper-IgM type 5 (HIGM5), Inclusion Body Myositis (IBM), polymyositis with mitochondrial pathology, IM-Mito, granulomatous myopathies with anti-mitochondrial antibodies, necrotizing myopathy with pipestem capillaries, myopathy with deficient chondroitin sulfate C in skeletal muscle connective tissue, benign acute childhood myositis, idiopathic orbital myositis, masticator myopathy, hemophagocytic lymphohistiocytosis, infection-associated myositis, Facioscapulohumeral dystrophy (FSH), familial idiopathic inflammatory myopathy, Schmidt Syndrome (Diabetes mellitus, Addison disease, Myxedema), TNF receptor-associated Periodic Syndrome (TRAPS), focal myositis, autoimmune fasciitis, Spanish toxic oil-associated fasciitis, Eosinophilic fasciitis, Macrophagic myofasciitis, Graft-vs-host disease fasciitis, Eosinophilia-myalgia Syndrome, perimyositis, isovaleric acidemia (IVA), Kearnes-Sayre Syndrome (KSS), 2-oxoadipic aciduria, 2-aminoadipic aciduria, Limb-girdle Muscular Dystrophy Syndromes,

leukodystrophy, Maple syrup urine disease (MSUD), 3-Methylcrotonyl-CoA carboxylase (MCC), Methylmalonic aciduria (MMA), Miller Syndrome, Mitochondrial DNA Depletion Syndrome-2 (MTDPS2), spinal muscular atrophy syndrome, rigid spine syndrome, severe myopathy with motor regression, Mitochondrial DNA Depletion Syndrome-3, MELAS Syndrome, camptocormia, MNGIE, MNGIM Syndrome, Menkes Disease, Occipital Horn Syndrome, X-linked distal spinal muscular atrophy-3 (SMAX3), methemoglobinemia, MERRF, progressive external ophthalmoplegia with myoclonus, deafness and diabetes (DD), multiple symmetric lipomatosis, Myopathy with Episodic high Creatine Kinase (MIMECK), Epilepsia Partialis Continua, malignant hyperthermia syndromes, glycogen metabolic disorders, fatty acid oxidation and lipid metabolism disorders, medication-, drug- or toxin-induced myoglobinuria, mitochondrial disorder-associated myoglobinuria, hypokalemic myopathy and rhabdomyolysis, muscle trauma-associated myoglobinuria, ischemia-induced myoglobinuria, infection-induced myoglobinuria, immune myopathies associated with myoglobinuria, Myopathy, lactic acidosis, and sideroblastic anemia (MLASA), infantile mitochondrial myopathy due to reversible COX deficiency (MMIT), Myopathy, Exercise intolerance, Encephalopathy and Lactic acidemia Syndrome, myoglobinuria and exercise intolerance syndrome, exercise intolerance, proximal weakness ± myoglobinuria syndrome, encephalopathy and seizures syndrome, septo-optic dysplasia, exercise intolerance ± mild weakness, myopathy ± exercise intolerance, growth or CNS disorder, maternally-inherited mitochondrial myopathies, myopathy with lactic acidosis, myopathy with rhabdomyolysis, Myopathy with cataract and combined respiratory chain deficiency, myopathy with abnormal mitochondrial translation, Fatigue Syndrome, myopathy with extrapyramidal movement disorders (MPXPS), glutaric aciduria II (MADD), primary CoQ10 deficiency-1 (COQ10D1), primary CoQ10 deficiency-2 (COQ10D2), primary CoQ10 deficiency-3 (COQ10D3), primary CoQ10 deficiency-5 (COQ10D5), secondary CoQ10 deficiency, autosomal dominant mitochondrial myopathy, myopathy with focal depletion of mitochondria, mitochondrial DNA breakage syndrome (PEO + Myopathy), lipid type mitochondrial myopathy, multiple symmetric lipomatosis (MSL), N-acetylglutamate synthase (NAGS) deficiency, Nephronophthisis (NPHP), ornithine transcarbamylase (OTC) deficiency, neoplasms, NARP Syndrome, paroxysmal nonkinesigenic dyskinesia (PNKD), sporadic PEO, maternally-inherited PEO, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-3 (PEOA3), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-2 (PEOA2), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-1 (PEOA1), PEO+ demyelinating

neuropathy, PEO + hypogonadism, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-4 (PEOA4), distal myopathy, cachexia & PEO, autosomal dominant progressive external ophthalmoplegia-6 (PEOA6), PEO + Myopathy and Parkinsonism, autosomal recessive progressive external ophthalmoplegia (PEOB), Mitochondrial DNA Depletion Syndrome-11 (MTDPS11), PEO with cardiomyopathy, PEPCK deficiency, Perrault Syndromes (PRLTS), propionic acidemia (PA), pyruvate carboxylase deficiency, pyruvate dehydrogenase E1-alpha deficiency (PDHAD), pyruvate dehydrogenase E1-beta deficiency (PDHBD), dihydrolipoamide dehydrogenase (DLD) deficiency, pyruvate dehydrogenase phosphatase deficiency, pyruvate dehydrogenase E3-binding protein deficiency (PDHXD), mitochondrial pyruvate carrier deficiency (MPYCD), Schwartz-Jampel Syndrome type 1 (SJS1), selenium deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency, succinyl CoA:3-oxacid CoA transferase (SCOT) deficiency, Stuve-Wiedemann Syndrome (STWS), thrombocytopenia (THC), Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), Vitamin D-dependent rickets type 1A (VDDR1A), Wilson's disease, Zellweger Syndrome (PBD3A), arsenic trioxide myopathy, myopathy and neuropathy resulting from nucleoside analogues, germanium myopathy, Parkinsonism and mitochondrial Complex I neurotoxicity due to trichloroethylene, valproate-induced hepatic failure, neurodegeneration with brain iron accumulation-4 (NBIA4), Complex I deficiency, Complex II deficiency, Complex III deficiency, Complex IV deficiency, Complex V deficiency, Cytochrome c oxidase (COX) deficiency, combined complex I, II, IV, ± V deficiency, combined complex I, II, and III deficiency, combined oxidative phosphorylation deficiency-1 (COXPD1), combined oxidative phosphorylation deficiency-2 (COXPD2), combined oxidative phosphorylation deficiency-3 (COXPD3), combined oxidative phosphorylation deficiency-4 (COXPD4), combined oxidative phosphorylation deficiency-6 (COXPD6), combined oxidative phosphorylation deficiency-7 (COXPD7), combined oxidative phosphorylation deficiency-9 (COXPD9), combined oxidative phosphorylation deficiency-11 (COXPD11), combined oxidative phosphorylation deficiency-12 (COXPD12), combined oxidative phosphorylation deficiency-13 (COXPD13), combined oxidative phosphorylation deficiency-15 (COXPD15), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-19 (COXPD19), combined oxidative phosphorylation deficiency-20 (COXPD20), combined oxidative phosphorylation deficiency-21 (COXPD21), fumarase deficiency, HMG-CoA synthase-2 deficiency, hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (HUPRA) Syndrome, syndromic microphthalmia-7, pontocerebellar hypoplasia type 6

(PCH6), Mitochondrial DNA Depletion Syndrome-9 (MTDPS9P), and Sudden infant death Syndrome (SIDS).

[0053] As used herein, a "mitochondrial targeting moiety" is a moiety that facilitates translocation of a molecule into the mitochondria and is selected from the group consisting of alkyltriphenylphosphonium, tetraphenylphosphonium, tetraphenylarsonium, tribenzyl ammonium, phosphonium, polyarginine, polylysine, and delocalized lipophilic cations containing one to three carbimino, sulfimino, or phosphinimino units as described in Kolomeitsev *et al*, *Tet. Let*, Vol. 44, No. 33, 5795-5798 (2003). Suitable alkyltriphenylphosphonium moieties include those alkyltriphenylphosphonium moieties containing a C_1-C_6 straight chain alkylene group having from 1 to 6 carbons, such as a methylene, ethylene, propylene, or butylene group.

[0054] As used herein, the "modulation" of, or to "modulate," an energy biomarker means to change the level of the energy biomarker towards a desired value, or to change the level of the energy biomarker in a desired direction (*e.g.*, increase or decrease). Modulation can include, but is not limited to, normalization and enhancement as defined herein.

[0055] As used herein, the terms "normalization" of, or to "normalize," an energy biomarker is defined as changing the level of the energy biomarker from a pathological value towards a normal value, where the normal value of the energy biomarker can be 1) the level of the energy biomarker in a healthy person or subject, or 2) a level of the energy biomarker that alleviates one or more undesirable symptoms in the person or subject. That is, to normalize an energy biomarker which is depressed in a disease state means to increase the level of the energy biomarker towards the normal (healthy) value or towards a value which alleviates an undesirable symptom; to normalize an energy biomarker which is elevated in a disease state means to decrease the level of the energy biomarker towards the normal (healthy) value or towards a value which alleviates an undesirable symptom.

[0056] As used herein, the term "optical isomer" is equivalent to the term "enantiomer."

[0057] The terms ortho, meta and para apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

[0058] A "pharmaceutically acceptable salt", with respect to derivatives of the compounds defined by Formula I, II, and III refer to instances where the parent compound is modified by making acid or base salts thereof. Example of pharmaceutically acceptable salts include but are not limited to mineral or organic acid salts of basic residues such as amines; and alkali or organic salts of acidic residues such as carboxylic acids. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric acids; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fiunaric, tolunesulfonic, naphthalenesulfonic, methanesulfonic, ethane disulfonic, oxalic, and isethionic salts. The pharmaceutically acceptable salts of the compounds can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, such as non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile, or in a mixture of the two.

[0059] As used herein "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[0060] As used herein, the terms “polypeptide,” “peptide,” and “protein” are used interchangeably herein to mean a polymer comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres. Polypeptide refers to both short chains, commonly referred to as peptides, glycopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art.

[0061] As used herein, “prevention” or “preventing” of a disease or medical condition refers to a compound that, in a statistical sample, reduces the occurrence of the disease or medical condition in the treated sample relative to an untreated control sample, or delays the onset of one or more symptoms of the disease or medical condition relative to the untreated control sample.

[0062] "Prodrug", as used herein, refers to a pharmacological substance (drug) which is administered in an inactive (or significantly less active) form. Once administered, the prodrug is metabolized in the body (*in vivo*) into the active compound.

[0063] The terms "racemate", "racemic mixture" or "racemic modification" refer to a mixture of equal parts of enantiomers.

[0064] As used herein, the term “simultaneous” therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

[0065] As used herein, the term “separate” therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

[0066] As used herein, the term “sequential” therapeutic use refers to administration of at least two active ingredients at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

[0067] "Solvate", as used herein, refers to a compound which is formed by the interaction of molecules of a solute with molecules of a solvent.

[0068] As used herein, the term "stereoisomers" refers to compounds made up of the same atoms having the same bond order but having different three-dimensional arrangements of atoms which are not interchangeable. The three-dimensional structures are called configurations.

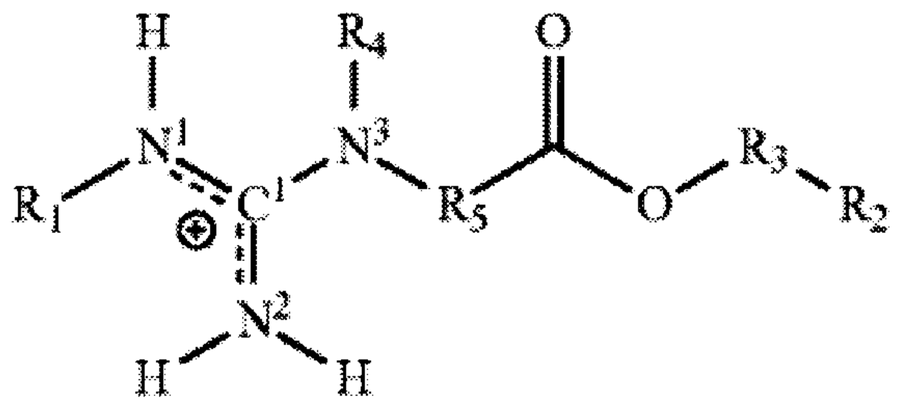
[0069] As used herein, a "synergistic therapeutic effect" refers to a greater-than-additive therapeutic effect which is produced by a combination of at least two agents, and which exceeds that which would otherwise result from the individual administration of the agents. For example, lower doses of one or more agents may be used in treating a disease or disorder, resulting in increased therapeutic efficacy and decreased side-effects.

[0070] "Treating" or "treatment" as used herein covers the treatment of a disease or medical condition described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or medical condition.

[0071] It is also to be appreciated that the various modes of treatment or prevention of medical diseases and conditions as described are intended to mean "substantial," which includes total but also less than total treatment or prevention, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

Mitochondrial-Targeted Creatine Compounds of the Present Technology

[0072] The present disclosure provides mitochondrial targeted-creatine compounds defined by the following structure:



(I)

wherein R_1 is H or phosphate and the double bond is between N^1 and C^1 or between N^2 and C^1;

R_2 is a mitochondrial targeting moiety selected from the group consisting of alkyltriphenylphosphonium, tetraphenylphosphonium, tetraphenylarsonium, tribenzyl ammonium, phosphonium, polyarginine, polylysine, and delocalized lipophilic

cations containing one to three carbimino, sulfimino, or phosphinimino units as described in Kolomeitsev *et al*, *Tet. Let*, Vol. 44, No. 33, 5795-5798 (2003);

R_3 is an alkyl, alkylaryl, alkylheteroaryl spacer group, a cleavable linker, or absent;

R_4 is H or an alkyl, aryl, or heteroaryl group; and

R_5 is alkyl, aryl, or heteroaryl; or

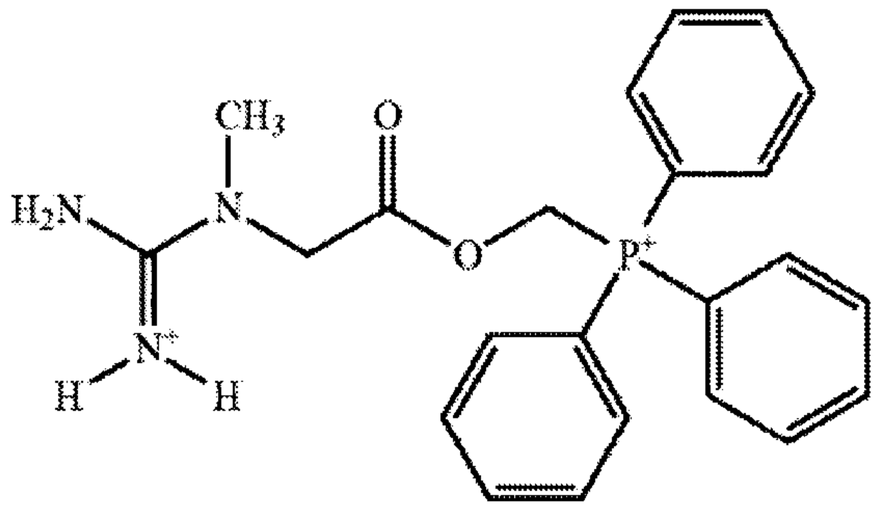
N^1, C^1, and N^3 together form a heterocyclic ring containing at least 5 atoms, wherein N^1, N^3, and R_1-R_5 are as defined above, or

N^3 and R_5 together form a heterocyclic ring containing at least four atoms; or

a pharmaceutically acceptable salt or prodrug thereof.

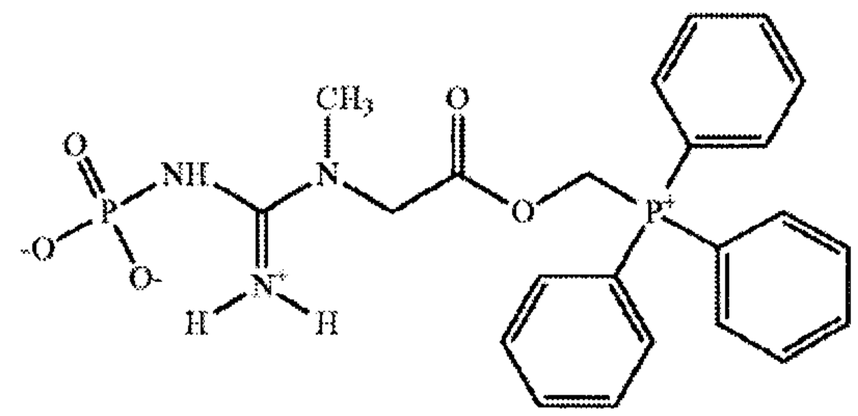
[0073] If the double bond is between C^1 and N^1, the positive charge typically will reside on N^1. If the double bond is between C^1 and N^2, the positive charge typically will reside on N^2.

[0074] In some embodiments, the mitochondrial targeted-creatine compounds are defined by Formula II and III:



(II)

(triphenylphosphonio)methyl N-[amino(iminio)methyl]-N-methylglycinate

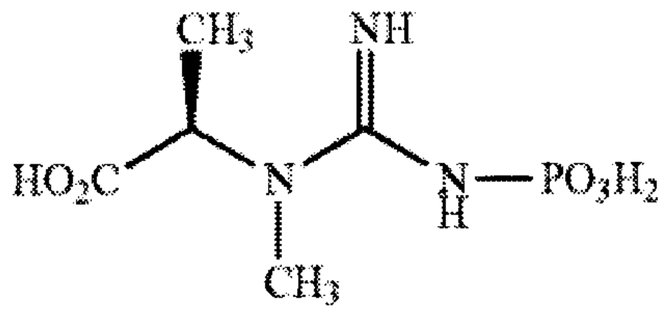


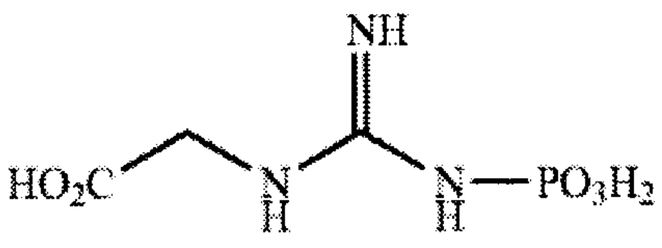
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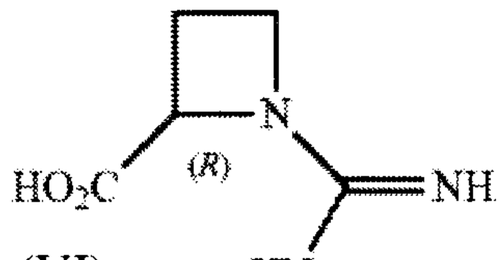
(triphenylphosphonio)methyl N-[amino(iminio)methyl]-N-methylglycinate phosphate.

[0075] The present disclosure also provides mitochondrial-targeted creatine analogs including, but not limited to the analogs shown below:

(IV)

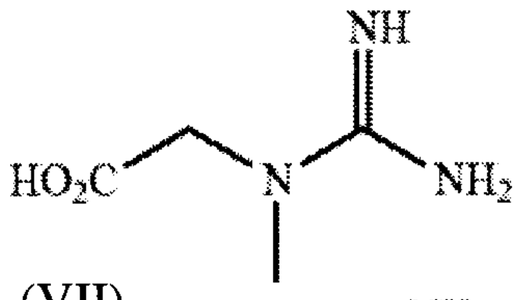


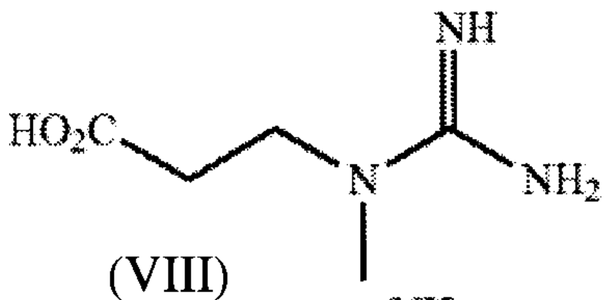




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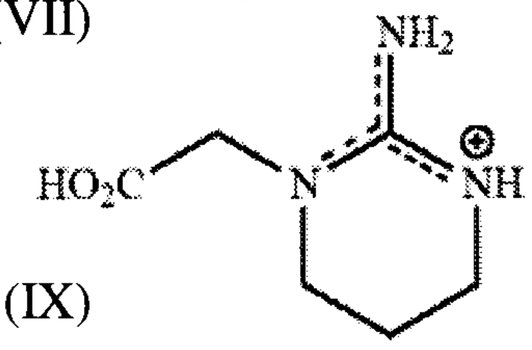
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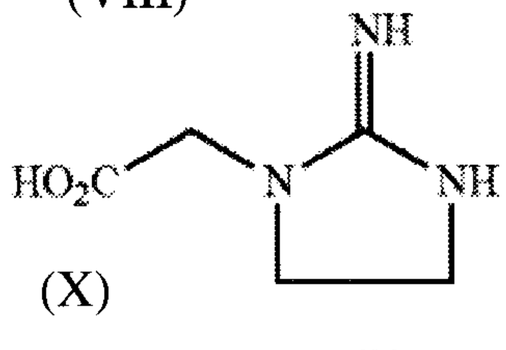




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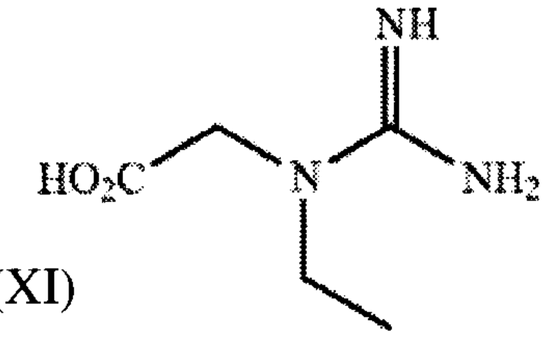
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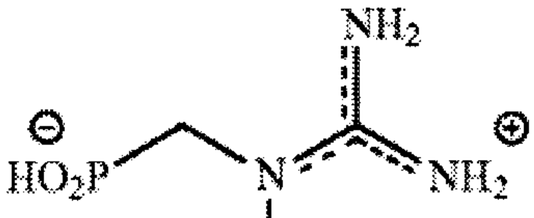




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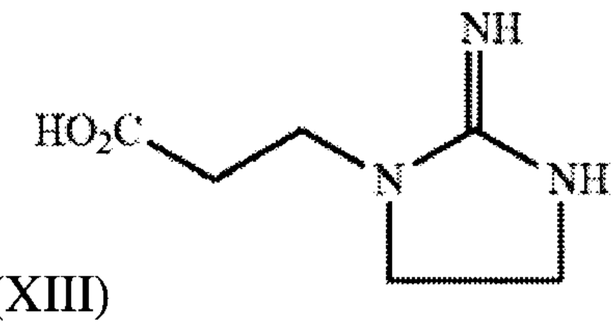
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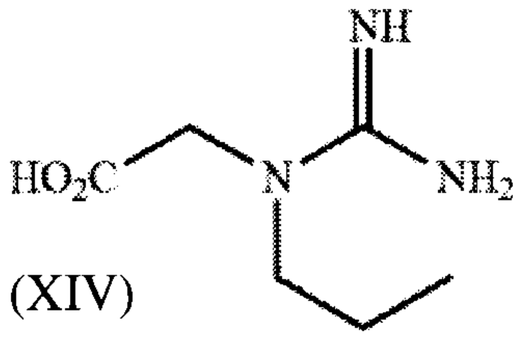




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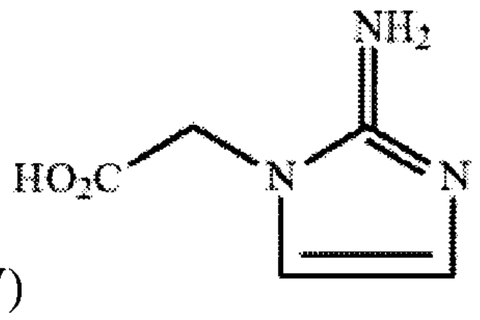
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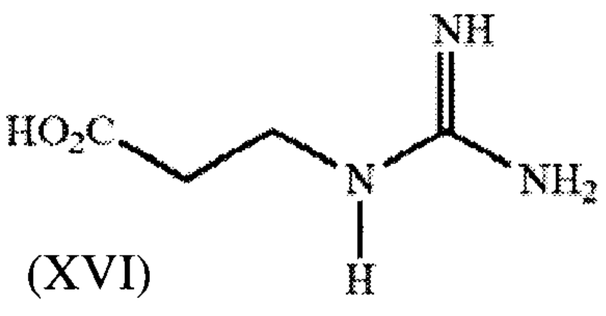




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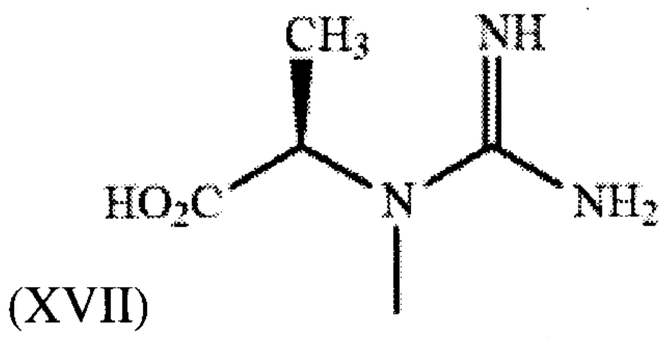
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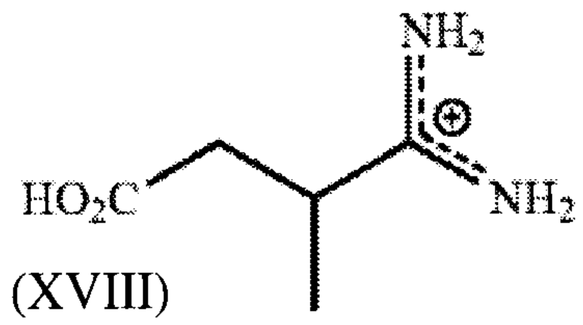


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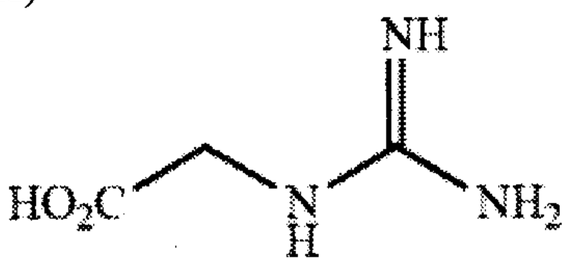
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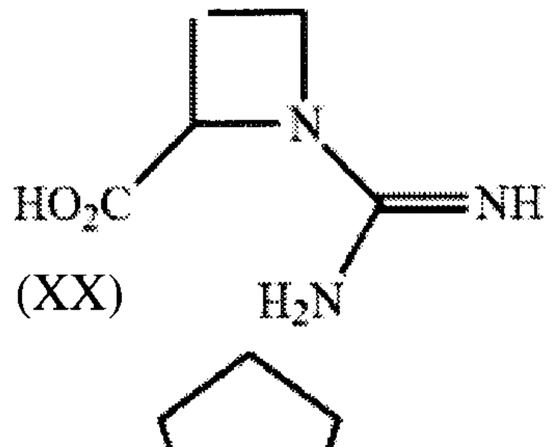
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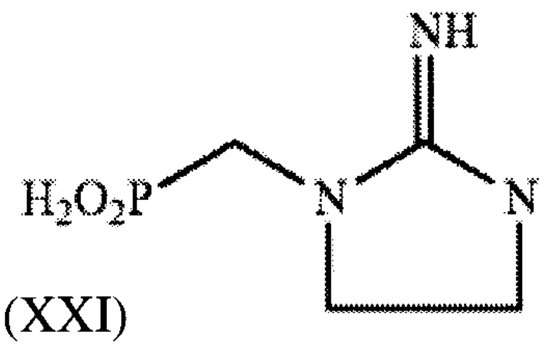
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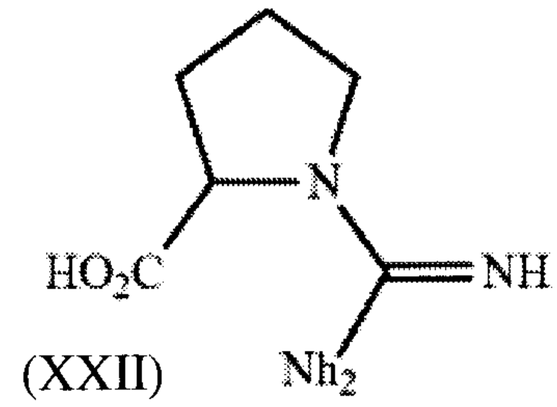
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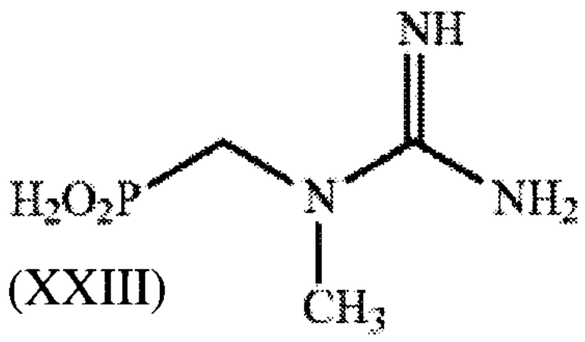
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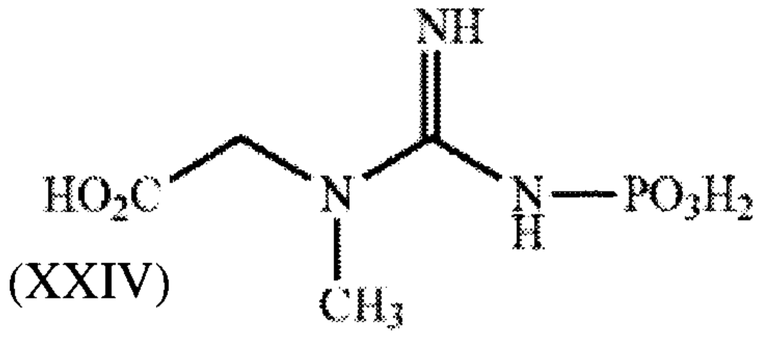
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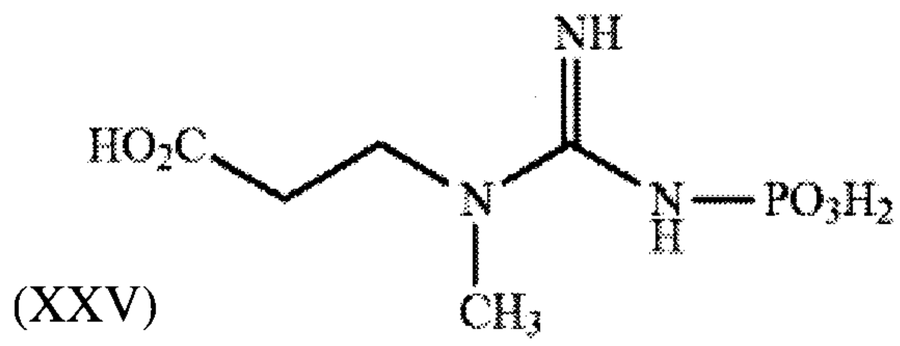
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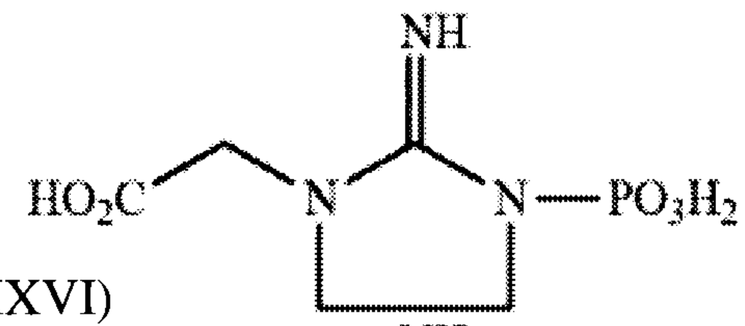
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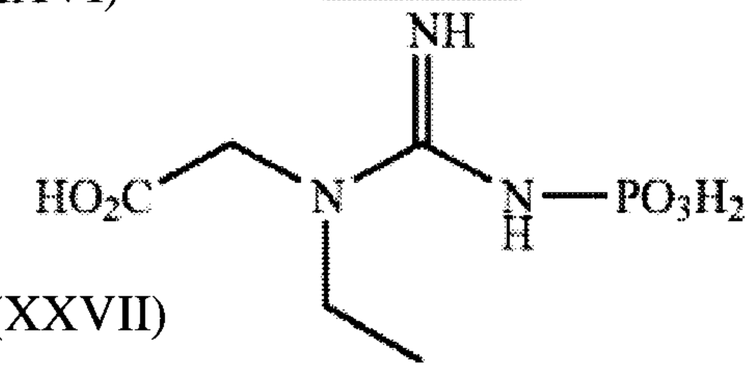
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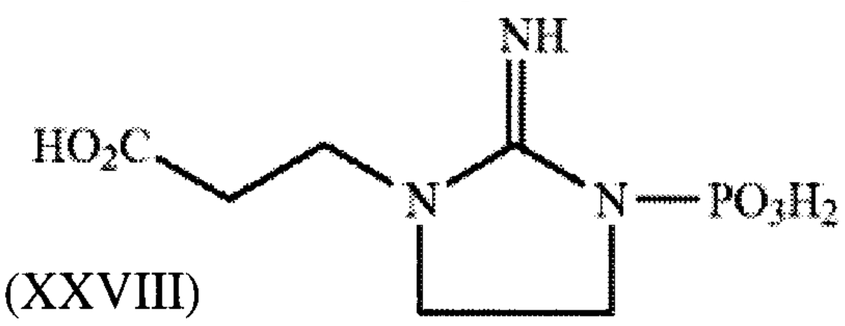
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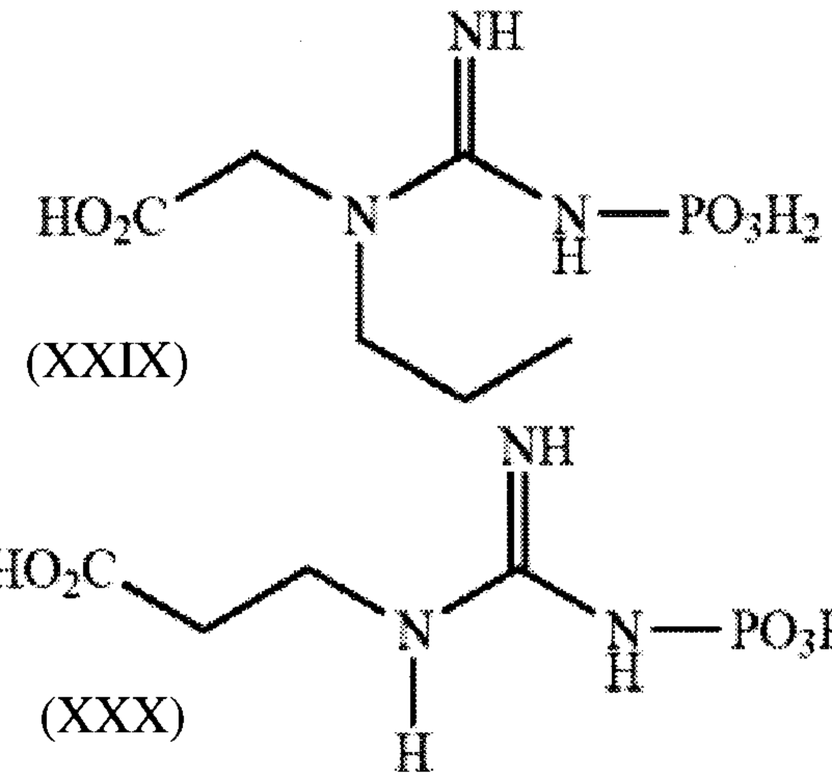
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(XXVII)



(XXVIII)



(XXIX)

(XXX)

wherein the mitochondrial targeting moiety is selected from the group consisting of alkyltriphenylphosphonium, tetraphenylphosphonium, tetraphenylarsonium, tribenzyl ammonium, phosphonium, polyarginine, polylysine, and delocalized lipophilic cations containing one to three carbimino, sulfimino, or phosphinimino units as described in Kolomeitsev *et al*, *Tet. Let*, Vol. 44, No. 33, 5795-5798 (2003). Generally, the mitochondrial targeting moiety is covalently coupled to creatine or a creatine analog via the carboxylic acid group.

[0076] Other exemplary creatine analogs that can be modified to include a mitochondrial targeting moiety as defined herein include, but are not limited to cyclocreatine (1-carboxymethyl-2-iminoimidazolidine), N-phosphorocreatine (N-phosphoryl creatine), cyclocreatine phosphate (3-phosphoryl-1-carboxymethyl-2-iminoimidazolidine), 1-carboxymethyl-2-aminoimidazole, 1-carboxymethyl-2,2-iminomethylimidazolidine, 1-carboxyethyl-2-iminoimidazolidine, N-ethyl-N-amidinoglycine and b-guanidinopropionic acid.

[0077] The compounds described herein may have one or more chiral centers and thus exist as one or more stereoisomers. Such stereoisomers can exist as a single enantiomer, a mixture of diastereomers or a racemic mixture.

[0078] The mitochondrial targeting moiety as defined herein can be linked directly to the disclosed creatine compounds or through a spacer group. Spacer groups include alkyl, alkylaryl or alkylheteroaryl spacer groups having a chain length from about C_1 to about C_{12}, or a subrange thereof such as C_1 to C_3, C_2 to C_4 *etc*. The spacer group is optionally substituted by one or more double and/or triple bonds. In certain aspects the total number of atoms in the alkylaryl and alkylheteroaryl groups is from about 6 to about 50.

[0079] The spacer group can optionally include a cleavable linkage or bond so that the mitochondrial targeting moiety as defined herein can be cleaved once the creatine compound of the present technology enters the mitochondrion. Representative cleavable linkages include, but are not limited to, amide bonds, ester bonds, and disulfide bonds.

Mitochondrial Diseases

[0080] Mitochondrial dysfunction plays a role both in the pathogenesis of late-onset neurodegenerative disorders, including Parkinson disease (PD), Huntington disease (HD), Alzheimer disease (AD), and amyotrophic lateral sclerosis (ALS), and in the pathogenesis of aging.

[0081] Mitochondrial diseases are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain. The mitochondrial respiratory chain is the only metabolic pathway in the cell that is under the dual control of the mitochondrial genome (mtDNA) and the nuclear genome (nDNA). While some mitochondrial disorders only affect a single organ (*e.g.*, the eye in Leber hereditary optic neuropathy [LHON]), many involve multiple organ systems and often present with prominent neurologic and myopathic features. Mitochondrial disorders may present at any age.

[0082] Mutations in mtDNA can be divided into those that impair mitochondrial protein synthesis *in toto* and those that affect any one of the 13 respiratory chain subunits encoded by mtDNA.

(i) *Heteroplasmy and threshold effect.* Each cell contains hundreds or thousands of mtDNA copies, which, at cell division, distribute randomly among daughter cells. In normal tissues, all mtDNA molecules are identical (homoplasmy). Deleterious mutations of mtDNA usually affect some but not all mtDNAs within a cell, a tissue, or an individual (heteroplasmy). The clinical expression of a pathogenic mtDNA mutation is largely determined by the relative proportion of normal and mutant mtDNA genomes in different tissues. A minimum critical number of mutant mtDNAs is required to cause mitochondrial dysfunction in a particular organ or tissue (threshold effect).

(ii) *Mitotic segregation.* At cell division, the proportion of mutant mtDNAs in daughter cells may shift and the phenotype may change accordingly. This phenomenon, called

mitotic segregation, explains how certain patients with mtDNA-related disorders may actually manifest different mitochondrial diseases at different stages of their lives.

(iii) *Maternal inheritance*. At fertilization, all mtDNA derives from the oocyte. Therefore, the mode of transmission of mtDNA and of mtDNA point mutations (single deletions of mtDNA are usually sporadic events) differs from Mendelian inheritance. A mother carrying a mtDNA point mutation will pass it on to all her children (males as well as females), but only her daughters will transmit it to their progeny. A disease expressed in both sexes but with no evidence of paternal transmission is strongly suggestive of a mtDNA point mutation.

[0083] Many individuals with a mutation of mtDNA display a cluster of clinical features that fall into a discrete clinical syndrome, such as the Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic weakness with ataxia and retinitis pigmentosa (NARP), or Leigh syndrome (LS). However, considerable clinical variability exists and many individuals do not fit neatly into one particular category, which is well-illustrated by the overlapping spectrum of disease phenotypes (including mitochondrial recessive ataxia syndrome (MIRAS) resulting from mutation of the nuclear gene POLG, which has emerged as a major cause of mitochondrial disease.

[0084] Disorders due to mutations in nDNA are more abundant not only because most respiratory chain subunits are nucleus-encoded but also because correct assembly and functioning of the respiratory chain require numerous steps, all of which are under the control of nDNA. These steps (and related diseases) include: (i) synthesis of assembly proteins; (ii) intergenomic signaling; (iii) mitochondrial importation of nDNA-encoded proteins; (iv) synthesis of inner mitochondrial membrane phospholipids; (v) mitochondrial motility and fission.

[0085] Common clinical features of mitochondrial disease – whether involving a mitochondrial or nuclear gene – include ptosis, external ophthalmoplegia, proximal myopathy and exercise intolerance, cardiomyopathy, sensorineural deafness, optic atrophy, pigmentary retinopathy, and diabetes mellitus. Common central nervous system findings are fluctuating encephalopathy, seizures, dementia, migraine, stroke-like episodes, ataxia, and spasticity. A

high incidence of mid- and late pregnancy loss is a common occurrence that often goes unrecognized.

[0086] Mitochondrial myopathies are characterized by excessive proliferation of normal- or abnormal-looking mitochondria in the muscle of patients with weakness or exercise intolerance. These abnormal fibers are referred to as "ragged red fibers" because the areas of mitochondrial accumulation appear purplish when contacted with the modified Gomori trichrome stain. Many patients with ragged red fibers often exhibit encephalomyopathy. However, the absence of ragged red fibers in a biopsy does not exclude a mitochondrial etiology.

[0087] *Diagnosis.* In some subjects, the clinical picture is characteristic of a specific mitochondrial disorder (*e.g.*, LHON, NARP, or maternally inherited Leigh Syndrome), and the diagnosis can be confirmed by identification of a mtDNA mutation on molecular genetic testing of DNA extracted from a blood sample. In many individuals, such is not the case, and a more structured approach is needed, including family history, blood and/or CSF lactate concentration, neuroimaging, cardiac evaluation, and molecular genetic testing for a mtDNA or nuclear gene mutation. Approaches to molecular genetic testing of a proband to consider are serial testing of single genes, multi-gene panel testing (simultaneous testing of multiple genes), and/or genomic testing (*e.g.*, sequencing of the entire mitochondrial genome exome or exome sequencing to identify mutation of a nuclear gene). In many individuals in whom molecular genetic testing does not yield or confirm a diagnosis, further investigation of suspected mitochondrial disease can involve a range of different clinical tests, including muscle biopsy for respiratory chain function.

[0088] A brief nonexhaustive summary of the various mitochondrial diseases or disorders is provided below.

Alexander Disease

[0089] In decreasing order of frequency, 3 forms of Alexander disease are recognized, based on age of onset: infantile, juvenile, and adult. Younger patients typically present with seizures, megalencephaly, developmental delay, and spasticity. In older patients, bulbar or pseudobulbar symptoms predominate, frequently accompanied by spasticity. The disease is progressive, with most patients dying within 10 years of onset. Imaging studies of the brain typically show cerebral white matter abnormalities, preferentially affecting the frontal region.

All 3 forms have been shown to be caused by autosomal dominant mutations in the GFAP (Glial fibrillary acidic protein) gene. Some patients with Alexander disease also exhibit mutations in NADH-Ubiquinone Oxidoreductase Flavoprotein 1 (NDUFV1).

[0090] Histologically, Alexander disease is characterized by Rosenthal fibers, homogeneous eosinophilic masses which form elongated tapered rods up to 30 microns in length, which are scattered throughout the cortex and white matter and are most numerous in the subpial, perivascular and subependymal regions. These fibers are located in astrocytes, cells that are closely related to blood vessels. Demyelination is present, usually as a prominent feature. A few cases have had hydrocephalus. Rosenthal fibers are commonly found in astrocytomas, optic nerve gliomas and states of chronic reactive gliosis, but they are especially conspicuous in Alexander disease. Rosenthal fibers found in this situation are typically the result of degenerative changes in the cytoplasm and cytoplasmic processes of astrocytic glial cell.

Alpers-Huttenlocher Disease (Alpers)

[0091] Mitochondrial DNA Depletion Syndrome-4A, also known as Alpers Syndrome, is an autosomal recessive disorder caused by mutations in POLG. Alpers is characterized by a clinical triad of psychomotor retardation, intractable epilepsy, and liver failure in infants and young children. Pathologic findings include neuronal loss in the cerebral gray matter with reactive astrocytosis and liver cirrhosis. The disorder is progressive and often leads to death from hepatic failure or status epilepticus before age 3 years. Symptoms include anoxic encephalopathy, fever, developmental delay, epilepsy, impaired central visual function, ataxia, sensory loss, neuronal loss, progressive liver failure, acute liver dysfunction precipitated by valproic acid, cirrhosis, hypotonia, dementia, vomiting, paralysis, stupor, jaundiced liver with fibrosis, inflammation and bile duct proliferation, and increased CSF protein and lactate.

[0092] Some affected individuals may show mild intermittent 3-methylglutaconic aciduria and defects in mitochondrial oxidative phosphorylation. Subjects with Alpers typically exhibit perturbations in pyruvate metabolism and NADH oxidation. For example, a subset of patients with mtDNA depletion and Alpers Syndrome show a global reduction in respiratory chain complex I, II/III, and IV activity and deficiency of mitochondrial DNA polymerase gamma activity. Neuropathologic changes characteristic of Alpers Syndrome, namely

laminar cortical necrosis, may also be seen in some patients with combined oxidative phosphorylation deficiency-14 (COXPD14) due to a mutation in the FARS2 gene.

Alpha-ketoglutarate Dehydrogenase Deficiency

[0093] Alpha-ketoglutarate dehydrogenase (AKDGH) deficiency is a disease of the tricarboxylic acid cycle (TCA cycle) that affects mitochondria metabolism. Alpha-ketoglutarate dehydrogenase is an enzyme of the TCA cycle that catalyzes the oxidation of alpha-ketoglutarate to succinyl CoA. Alpha-ketoglutarate dehydrogenase is one of 3 alpha-ketoacid dehydrogenases, the others being pyruvate dehydrogenase and branched-chain ketoacid dehydrogenase. The alpha-ketoglutarate dehydrogenase complex is a multi-enzyme complex consisting of three protein subunits: oxoglutarate dehydrogenase, also known as alpha-ketoglutarate dehydrogenase or E1k; dihydrolipoyl succinyltransferase, also known as DLST or E2k; and dihydrolipoyl dehydrogenase, also known as DLD or E3. AKDGH deficiency is associated with DLD deficiency, which is caused by a mutation in the DLD gene. AKDGH deficiency is characterized by encephalopathy and hyperlactatemia resulting in death in early childhood.

Frontotemporal Dementia and/or Amyotrophic Lateral Sclerosis (ALS-FTD)

[0094] ALS-FTD is an autosomal dominant late-onset (between 49 to 65 years) neurodegenerative disorder comprising frontotemporal dementia, cerebellar ataxia, myopathy, and motor neuron disease consistent with amyotrophic lateral sclerosis, caused by disruptions in the CHCHD10 gene. Clinical manifestations include progressive bulbar dysfunction, dementia, sensorineural deafness, extensor plantar responses, dysphagia, dysarthria, myopathy, and a frontal lobe syndrome. Muscle biopsies usually show ragged red fibers, cytochrome C oxidase (COX)-negative fibers, and mitochondrial DNA deletions; many patients also have combined mitochondrial respiratory chain deficiencies and fragmented mitochondrial networks in fibroblasts, all suggestive of mitochondrial dysfunction. Other features include signs of Parkinsonism, including akinesia and rigidity, sensorineural hypoacusis, and fatigue. Overexpression of the mutant CHCHD10 protein in HeLa cells results in fragmentation of the mitochondrial network as well as major ultrastructural abnormalities, thereby implicating a role for dysfunctional mitochondria in the pathogenesis of late-onset frontotemporal dementia with motor neuron disease.

Anemia

1. Sideroblastic Anemia with Spinocerebellar Ataxia

[0095] Sideroblastic anemia with spinocerebellar ataxia is caused by mutations in the ATP-binding cassette 7 (ABCB7) transporter, which mediates ATP-dependent transfer of solutes. ABCB7 is an inner mitochondrial membrane protein that contains 2 transmembrane domains that form a membranous pore and 2 cytosolic ATP-binding domains, which couple ATP binding to solute movement. Affected males exhibit a moderate hypochromic microcytic anemia with ring sideroblasts on bone marrow examination and raised free erythrocyte protoporphyrin levels and no excessive parenchymal iron storage in adulthood. Neurologic features include non-progressive ataxia or incoordination (age of onset at 1 year), accompanied by long motor tract signs (hyperactive deep tendon reflexes, positive Babinski sign, clonus) in young affected males. Heterozygous females may exhibit mild anemia, but not ataxia.

2. Sideroblastic Anemia, Pyridoxine-refractory

[0096] Pyridoxine-refractory sideroblastic anemia is an autosomal recessive disorder caused by a homozygous or compound heterozygous mutation in the SLC25A38 gene. In addition, a homozygous mutation in the GLRX5 gene has been identified in some patients with late-onset autosomal recessive pyridoxine-refractory sideroblastic anemia. Clinical features include severe microcytic hypochromic anemia, hepatosplenomegaly, jaundice, iron overload, and cirrhosis. Patients typically exhibit moderate erythroid expansion in the bone marrow, and increased iron staining both in erythroblasts and macrophages, with 28% ringed sideroblasts. In some cases, patients may show low levels of δ-aminolevulinic acid synthase in erythroblasts.

3. Growth Retardation, Amino aciduria, Cholestasis, Iron overload, Lactic acidosis, Early death (GRACILE) Syndrome

[0097] GRACILE Syndrome, an autosomal recessive disorder usually observed in Finnish and Turkish populations, is caused by disruptions in the BCSIL gene which is required for the expression of functional ubiquinol-cytochrome-c reductase (bc1) complex. Loss of BCSIL function results in tubulopathy, encephalopathy, and liver failure due to complex III deficiency. Clinical features include severe intrauterine growth retardation, fulminant lactic acidosis during the first days of life, Fanconi-type amino aciduria, spasticity, increased

tendon reflexes, and abnormalities in iron metabolism, including liver hemosiderosis. Affected infants fail to thrive, and die neonatally or in early infancy. Other BCS1L disorders include Björnstad Syndrome, Leigh Syndrome and mitochondrial complex III deficiency, nuclear type 1 (MC3DN1).

4. Anemia and Mitochondriopathy (COXPD18)

[0098] Anemia and mitochondriopathy, or combined oxidative phosphorylation deficiency 18 (COXPD18) is an autosomal recessive disorder caused by a homozygous or compound heterozygous mutation in the SFXN4. COXPD18 is characterized by intrauterine growth retardation, intellectual disability, dysmetria, tremor, muscular atrophy, hypotonia, visual impairment, speech delay, delayed motor skills, and lactic acidosis associated with decreased mitochondrial respiratory chain activity. Affected patients may also show hematologic abnormalities, mainly macrocytic anemia and hypersegmented neutrophils, and increased blood lactate and ammonia levels.

5. Thiamine-responsive Megaloblastic Anemia

[0099] Thiamine-responsive Megaloblastic Anemia Syndrome (TRMA), also known as Thiamine Metabolism Dysfunction Syndrome-1 (THMD1), can be caused by a homozygous mutation in the SLC19A2 gene, which encodes a thiamine transporter protein. Thiamine-responsive Megaloblastic Anemia Syndrome comprises megaloblastic anemia, diabetes mellitus, amino aciduria, and sensorineural deafness. Onset is typically between infancy and adolescence, but all of the cardinal findings are often not present initially. The anemia, and sometimes the diabetes, improves with high doses of thiamine. Other more variable features include optic atrophy, congenital heart defects, short stature, and stroke.

6. Pearson Syndrome

[0100] Pearson Syndrome is caused by a deletion in mitochondrial DNA and is characterized by sideroblastic anemia and exocrine pancreas dysfunction. With Pearson Syndrome, the bone marrow fails to produce white blood cells called neutrophils. The syndrome also leads to anemia, low platelet count, and aplastic anemia. Pearson Syndrome causes the exocrine pancreas to not function properly because of scarring and atrophy. Individuals with this condition have difficulty absorbing nutrients from their diet which leads to malabsorption. Infants with this condition generally do not grow or gain weight

[0101] Other clinical features are failure to thrive, pancytopenic crises, pancreatic fibrosis with insulin-dependent diabetes and exocrine pancreatic deficiency, muscle and neurologic impairment, malabsorption, steatorrhea, metabolic and lactic acidosis, and early death. The few patients who survive into adulthood often develop symptoms of Kearns-Sayre Syndrome.

Ataxia

[0102] Ataxia is defined as the presence of abnormal, uncoordinated movements. Defects affecting either the mitochondrial or nuclear genomes can cause mitochondrial dysfunction, resulting in mitochondrial ataxia. Ataxia associated with mtDNA defects typically manifests as part of a multisystem, multisyndrome disorder.

Maternally Inherited Ataxias

1. HAM Syndrome

[0103] HAM (Hearing loss, Ataxia, Myoclonus) is a maternally-inherited syndrome characterized by a combination of sensorineural hearing loss, ataxia, and myoclonus observed in a large kindred from Sicily. Hearing loss is the most prevalent and sometimes the only symptom found in family members. HAM Syndrome is associated with the presence of a C7472 insertion mutation in mtDNA regions encompassing the tRNA genes. This particular insertion is found in the MT-TS1 gene (nucleotides 7445-7516), which encodes the mitochondrial tRNA for serine (UCN). The insertion adds a seventh cytosine to a six-cytosine run that is part of the mitochondrial tRNASer(UCN) gene. Conformational analyses demonstrate that this mutation likely alters the clover leaf secondary structure of tRNASer/(UCN).

2. Ataxia, Cataract, and Diabetes Syndrome and MELAS/MERRF Overlap Syndrome

[0104] Mutations in the mitochondrial MT-TS2 gene (nucleotides 12207-12265), which encodes the mitochondrial tRNA for serine (AGY) are associated with the development of maternally-inherited cerebellar ataxia, cataract, and diabetes mellitus. In particular, these phenotypes are associated with a C-to-A transversion at position 12258. It is thought that this mutation alters a highly conserved basepair in the acceptor stem of the tRNA(Ser) molecule, which would affect aminoacylation of the tRNA thereby altering the function of the tRNA for serine and reducing the accuracy of mitochondrial translation.

[0105] Other mutations in the same gene cause MELAS/MERRF Overlap Syndrome. One such mutation is a heteroplasmic 12207G-A transition in the MT-TS2 gene. Upon examination, skeletal muscle biopsies revealed ragged red fibers, significant pleomorphic mitochondrial proliferation, and complex I deficiency. The 12207G-A mutation occurs in a region involved in the formation of the acceptor stem of the tRNA molecule. Individuals with MELAS/MERRF Overlap Syndrome experience a combination of the signs and symptoms of both disorders as described above.

3. Cytochrome c Oxidase Deficiency

[0106] Cytochrome c oxidase (COX) deficiency is a mitochondrial disorder caused by a lack of COX. Cytochrome c oxidase, also known as complex IV, is the terminal enzyme of the mitochondrial respiratory chain located within the mitochondrial inner membrane. Complex IV is composed of 13 polypeptides. Subunits I, II, and III (MTCO1, MTCO2, and MTCO3) are encoded by mtDNA, while subunits IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, and VIII are nuclear encoded. Because COX is encoded by both nuclear and mitochondrial genes, COX deficiency can be inherited in either an autosomal recessive or maternal pattern. A G-to-A transition at nucleotide 6480 of the MTCO1 gene, which encodes cytochrome c oxidase subunit I, is associated with sensorineural hearing loss, ataxia, myoclonic epilepsy, and mental retardation. The signs and symptoms of COX deficiency typically manifest before two years of age, but can appear later in mildly affected individuals.

[0107] Another form of cytochrome c oxidase deficiency results from mutations in the MTCO2 gene, which encodes cytochrome c oxidase subunit II. A T-to-C transition at nucleotide 7587 of the MT-CO2 gene is associated with ataxia, distal weakness, retinopathy, and optic atrophy.

Recessive ataxia syndromes

1. Infantile Cerebellar-retinal Degeneration

[0108] Infantile cerebellar-retinal degeneration (ICRD), also known as mitochondrial aconitase deficiency, is associated with a Ser112Arg mutation in the nuclear ACO2 gene, which encodes mitochondrial aconitase. Aconitase catalyzes the isomerization of citrate to isocitrate *via* cis-aconitate in the second step of the TCA cycle. ICRD is a severe autosomal recessive neurodegenerative disorder characterized by onset between two and six months of age of truncal hypotonia, athetosis, seizures, and ophthalmologic abnormalities, including

optic atrophy and retinal degeneration. Individuals with ICRD exhibit profound psychomotor retardation and progressive cerebral and cerebellar degeneration.

2. Charlevoix-Saguenay Spastic Ataxia

[0109] Charlevoix-Saguenay spastic ataxia, also known as autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), is caused by a homozygous or compound heterozygous mutation in the SACS gene encoding the sacsin protein. Research suggests that sacsin may interact with the heat shock protein 70 (Hsp70) chaperone machinery, which plays an important role in protein folding and the cellular response to aggregation-prone mutant proteins associated with neurodegenerative diseases. Mutations in the SACS gene lead to the production of unstable sacsin protein that fails to function normally. ARSACS is a complex neurodegenerative disorder characterized by the progressive degeneration of the cerebellum and spinal cord and early childhood onset of cerebellar ataxia, pyramidal tract signs, peripheral neuropathy, retinal changes, and, in some cases, cognitive decline.

3. Primary Coenzyme Q10 Deficiency-1

[0110] Primary coenzyme Q10 deficiency-1 (COQ10D1) is an autosomal recessive disorder caused by a homozygous or compound heterozygous mutation in the COQ2 gene, which encodes COQ2, or parahydroxybenzoic-polyprenyltransferase. COQ2 catalyzes one of the final reactions in the biosynthesis of CoQ10, the prenylation of parahydroxybenzoate with an all-trans polyprenyl group. Coenzyme Q10 (CoQ10), or ubiquinone, functions as an electron carrier critical for electron transfer by the mitochondrial inner membrane respiratory chain, and is a lipid-soluble antioxidant. Primary CoQ10 deficiency-1 disorder is associated with five major phenotypes, including: an encephalomyopathic form with seizures and ataxia; a multisystem infantile form with encephalopathy, cardiomyopathy and renal failure; a predominantly cerebellar form with ataxia and cerebellar atrophy; Leigh Syndrome with grown retardation; and an isolated myopathic form.

4. Ataxia with Oculomotor Apraxia Type 1

[0111] Ataxia with oculomotor apraxia (AOA) comprises a group of autosomal recessive disorders characterized by ataxia, oculomotor apraxia, and choreoathetosis. AOA includes ataxia telangiectasia (AT), ataxia telangiectasia like disorder (ATLD), ataxia oculomotor apraxia type 1 (AOA1), and ataxia oculomotor apraxia type 2 (AOA2). AOA1, also known as ataxia, early-onset, with oculomotor apraxia and hypoalbumineria, is characterized by

early-onset cerebellar ataxia, oculomotor apraxia, hypoalbumineria, hypercholesterolemia, and late axonal sensorimotor neuropathy. AOA1 is caused by mutations in the APTX gene, which encodes aprataxin, a member of the histidine triad (HIT) superfamily, members of which have nucleotide-binding and diadenosine polyphosphate hydrolase activities. Aprataxin is a DNA-binding protein involved in single-strand DNA break repair, double-strand DNA break repair, and base excision repair. Mutations in APTX result in the production of an unstable aprataxin protein that is quickly degraded in the cell. Nonfunctional aprataxin leads to an accumulation of breaks in DNA, particularly in the neurocytes of the cerebellum where DNA repair is critical.

5. Autosomal Recessive Spinocerebellar Ataxia-9

[0112] Autosomal recessive spinocerebellar ataxia-9 (SCAR9), also known as coenzyme Q10 deficiency-4 (COQ10D4), is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the COQ8 gene. SCAR9 is characterized by childhood-onset of cerebellar ataxia and exercise intolerance. Patients manifest gait ataxia, cerebellar atrophy with slow progression. Additional features include variable seizures, mild mental impairment, brisk tendon reflexes, and Hoffmann sign.

6. Ataxia, Pyramidal Syndrome, and Cytochrome Oxidase Deficiency

[0113] A homozygous missense mutation in COX20, also known as FAM36A, causes impaired cytochrome c oxidase assembly and is associated with ataxia and muscle hyptonia. Additional clinical symptoms include oligohydramnios and growth retardation during pregnancy, low birth weight, delayed speech development, pyramidal signs, short stature, mildly elevated serum and cerebrospinal fluid lactate levels, and myocyte complex IV deficiency. The mutation has been identified as a homozygous c.154A-C transversion in exon 2 of the COX20 gene, resulting in a T52P substitution at a highly conserved residue at the interface between the inner-membrane embedded region and the predicted mitochondrial matrix-localized loop fragment. The COX2 gene encodes cytochrome c oxidase protein 20, which plays a role in the assembly of mitochondrial complex IV and interacts with cytochrome c oxidase subunit II.

7. Friedreich's Ataxia

[0114] Friedreich's ataxia is an autosomal recessive neurodegenerative disorder caused by a mutation in the FXN gene, which encodes frataxin. Frataxin is a nuclear-encoded

mitochondrial iron chaperone that is localized to the inner mitochondrial membrane and involved in iron-sulfur biogenesis and heme biosynthesis. The most common molecular abnormality is a GAA trinucleotide repeat expansion in intron 1 of the FXN gene. Whereas normal individual have 5 to 30 GAA repeat expansions, individuals affected with Friedreich's ataxia have from 70 to more than 1,000 GAA triplets.

[0115] Friedreich's ataxia is characterized by progressive gait and limb ataxia with associated limb muscle weakness, absent lower limb reflexes, extensor plantar responses, dysarthria, and decreased vibratory sense and proprioception. Other features include visual defects, scoliosis, pes cavus, and cardiomyopathy. Onset typically occurs in the first or second decade. Affected individuals who develop Friedreich's ataxia between ages 26 and 39 are considered to have late-onset Friedreich's ataxia (LOFA). When the signs and symptoms begin after age 40 the condition is called very late-onset Friedreich's ataxia (VLOFA). LOFA and VLOFA usually progress more slowly than typical Friedreich's ataxia.

8. Infantile Onset Spinocerebellar Ataxia

[0116] Infantile onset spinocerebellar ataxia (IOSCA), also known as Mitochondrial DNA Depletion Syndrome-7, is an autosomal recessive severe neurodegenerative disorder caused by a homozygous or compound heterozygous mutation in the nuclear-encoded C10ORF2 gene, which encodes the twinkle and twinky proteins. Twinkle is a mitochondrial protein involved in mtDNA metabolism. The C10ORF2 gene mutations that cause IOSCA interfere with the function of twinkle resulting in mtDNA depletion. IOSCA is associated with the following C10ORF2 mutations: P83S/R463W; Y508C/A318T; Y508C/R29X; T451I/T451I; c.1460C-T (T487I)/c.1485-1G-A; and 1472C-T.

[0117] IOSCA is characterized by hypotonia, ataxia, ophthalmoplegia, hearing loss, seizures, sensory axonal neuropathy, reduced mental capacity, and mtDNA depletion in the brain and liver. Individuals affected with IOSCA often develop autonomic nervous system disorders and experience excessive sweating, incontinence, and constipation.

9. Leukoencephalopathy with Brain Stem and Spinal Cord Involvement and Lactate Elevation

[0118] Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) is an autosomal recessive disorder that can be caused by homozygous or

compound heterozygous mutations in the gene encoding mitochondrial aspartyl-tRNA synthetase (DARS2). The mutation results in reduced aspartyl-tRNA synthetase activity.

[0119] LBSL is defined by a highly characteristic constellation of abnormalities observed by magnetic resonance imaging and spectroscopy. These include a pattern of inhomogeneous cerebral white matter abnormalities, selective involvement of brainstem and spinal tracts, and increased lactate in the abnormal white matter. Affected individuals develop slowly progressive cerebellar ataxia, spasticity, and dorsal column dysfunction, sometimes with a mild cognitive deficit or decline. Onset typically occurs between three and fifteen years of age.

10. Autosomal Recessive Spastic Ataxia-3

[0120] Autosomal recessive spastic ataxia-3 (SPAX3), also known as autosomal recessive spastic ataxia with leukoencephalopathy (ARSAL), is caused by homozygous or compound heterozygous complex genomic rearrangements involving the MARS2 gene, which encodes mitochondrial methionyl-tRNA synthetase (mtMetRS), a protein localized to the mitochondrial matrix. The protein shares a high degree of identity with methionyl-tRNA synthetases from other mammals. Mutations in MARS2 result in reduced protein levels. Some affected individuals have a heterozygous 268-bp deletion in the MARS2 gene, resulting in a frameshift and premature termination (c.681del268bpfs236Ter). Other affected individuals have duplications of the MARS2 gene.

[0121] SPAX3 is a progressive disease characterized by ataxia, dysarthria, horizontal nystagmus, spasticity, hyperreflexia, urinary urgency, scoliosis, dystonia, cognitive impairment, optic atrophy, cataract, hearing loss, cerebellar atrophy, cortical atrophy, leukoencephalopathy, and complex I deficiency. The disease usually manifests between birth and fifty-nine years of age.

11. MIRAS and SANDO

[0122] Mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO) are disorders that fall under a group of conditions known as the ataxia neuropathy spectrum. Ataxia neuropathy spectrum is caused by mutations in the POLG gene, and, rarely, the C10ORF2 gene. The POLG gene encodes DNA polymerase gamma, which functions in the replication of mitochondrial DNA. The POLG protein is composed of a C-terminal polymerase domain and an amino-terminal

exonuclease domain. The exonuclease domain increases the fidelity of mitochondrial DNA replication by conferring a proofreading activity to the enzyme.

[0123] MIRAS is associated with W748S and E1143G cis, and homozygous W748S or A467T POLG1 mutations. Clinical symptoms of MIRAS include ataxia, polyneuropathy, reduced muscle strength, cramps, epilepsy, cognitive impairment, athetosis, tremor, obesity, eye movement disorders, cerebellar atrophy, white matter changes, muscle denervation, and, rarely, mitochondrial alterations. MIRAS onset typically occurs between five and thirty-eight years of age.

[0124] SANDO is commonly associated with a compound heterozygous or homozygous A467T mutation. Other mutations associated with SANDO include N468D, G517V, G737R, R1138C, and E1143G missense mutations. SANDO is a progressive disease characterized by neuropathy causing sensory loss, variable alterations in strength, ataxia, absent or reduced tendon reflexes, ptosis, ophthalmoplegia, dysarthria, facial weakness, myoclonic epilepsy, and depression. Additional features include elevated serum and cerebrospinal fluid lactate levels, spinocerebellar and dorsal column tract degeneration, thalamic lesions, cerebellar atrophy or white matter changes, multiple mtDNA deletions, ragged red fibers, loss of myelinated and unmyelinated axons, posterior column atrophy, dorsal root ganglia neuron loss, reduced mtDNA number in affected neurons, and reduced activity of mitochondrial complexes I and IV.

12. Mitochondrial Spinocerebellar Ataxia and Epilepsy

[0125] Mitochondrial spinocerebellar ataxia and epilepsy (MSCAE) is a disorder comprising spinocerebellar ataxia, peripheral neuropathy, and epilepsy. Onset typically occurs during second and third decades and can be with ataxia or epilepsy, but all patients with MSCAE will develop ataxia if they survive, while only approximately 80% will develop epilepsy. Other clinical features include migraine, myoclonus or myoclonic seizures, high T2 signal in the thalamus, occipital cortex, and cerebellum, cerebellar atrophy, enlarged olives, stroke-like lesions, mtDNA depletion in neurons, and progressively reduced complex I. Several POLG1 mutations are associated with this disorder, the most common being the c.1399G-A that gives p.A467T, the c.2243G-C giving the p.W748S, and the Gln497His mutation.

13. Spastic Ataxia with Optic Atrophy

[0126] Spastic ataxia with optic atrophy (SPAX4) is a slowly progressive autosomal recessive neurodegenerative disease characterized by cerebellar ataxia, spastic paraparesis, dysarthria, and optic atrophy. SPAX4 is associated with mutations affecting the MTPAP gene, resulting in a defect of mitochondrial mRNA maturation. One particular mutation is a homozygous N478D missense mutation. The MTPAP gene encodes a polymerase that is a member of the DNA polymerase type-B-like family. The enzyme synthesizes the 3′ poly(A) tail of mitochondrial transcripts and plays a role in replication-dependent histone mRNA degradation. Affected individuals exhibit decreased poly(A) tail length of mitochondrial transcripts including those for COX1 and RNA14.

14. Mitochondrial Complex I Deficiency

[0127] Mitochondrial complex I deficiency (MT-C1D) is a disorder of the mitochondrial respiratory chain associated with mutations in the NUBPL gene. The NUBPL gene encodes a member of the Mrp/NBP35 ATP-binding proteins family. The encoded protein is required for the assembly of complex I (NADH dehydrogenase), located in the mitochondrial inner membrane. MT-C1D is characterized by a wide variety of clinical manifestations ranging from lethal neonatal disease to adult-onset neurodegenerative disorders including macrocephaly with progressive leukodystrophy, non-specific encephalopathy, cardiomyopathy, myopathy, liver disease, Leigh Syndrome, LHON, and some forms of Parkinson's disease.

15. Progressive External Ophthalmoplegia with Mitochondrial DNA Deletions Autosomal Dominant Type 5

[0128] Progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5) can be either an autosomal dominant or recessive disorder caused by mutations in the nuclear-encoded RRM2B gene. Recessive inheritance of PEOA5 is associated with homozygous or compound heterozygous missense variations in the RRM2B gene. PEOA5 is characterized by progressive weakness of ocular muscles and levator muscle of the upper eyelid. In a minority of cases, it is associated with skeletal myopathy, which predominantly involves axial or proximal muscles and which causes abnormal fatigability. Ragged red fibers and atrophy are found on muscle biopsy. Additional symptoms may include cataracts, hearing loss, sensory axonal neuropathy, ataxia, depression, hypogonadism, and Parkinsonism.

16. Mitochondrial Complex III Deficiency Nuclear Type 2

[0129] Mitochondrial complex III deficiency nuclear type 2 (MC3DN2) is an autosomal recessive severe neurodegenerative disorder caused by a homozygous or compound heterozygous mutation in the nuclear-encoded TTC19 gene. The TTC19 gene encodes tetratricopeptide repeat protein 19, a subunit of mitochondrial respiratory chain complex III, which transfers electrons from coenzyme Q to cytochrome c. This electron transfer contributes to the extrusion of protons across the inner mitochondrial membrane and contributes to the mitochondrial electrochemical potential. Mutations in the TTC19 gene include Leu219X and Gln173X nonsense mutations.

[0130] MC3DN2 presents in childhood, but may show later onset, even in adulthood. Affected individuals have motor disability, with ataxia, apraxia, dystonia, and dysarthria, associated with necrotic lesions throughout the brain. Most patients also have cognitive impairment and axonal neuropathy and become severely disabled later in life. The disorder may present clinically as spinocerebellar ataxia or Leigh Syndrome, or with psychiatric disturbances. Complex III deficiency is observed on muscle biopsy.

17. Episodic Encephalopathy due to Thiamine Pyrophosphokinase Deficiency

[0131] Episodic encephalopathy due to thiamine pyrophosphokinase deficiency, also known as Thiamine Metabolism Dysfunction Syndrome-5 (THMD5), is an autosomal recessive thiamine metabolism disorder caused by a homozygous or compound heterozygous mutation in the TPK1 gene. The TPK1 gene encodes thiamine pyrophosphokinase (TPK), an enzyme involved in the regulation of thiamine metabolism. TPK catalyzes the conversion of thiamine, a form of vitamin B1, to thiamine pyrophosphate (TPP). Thymine pyrophosphate is an active cofactor for enzymes involved in glycolysis and energy production, including transketolase, pyruvate dehydrogenase, and alpha-ketoglutarate dehydrogenase.

[0132] Onset of episodic encephalopathy due to thiamine pyrophosphokinase deficiency typically occurs between one and four years of age. Affected individuals present with a highly variable phenotype characterized by progressive neurologic dysfunction manifested as ataxia, dystonia, spasticity, inability to walk, mildly delayed developments, and increased serum and cerebrospinal fluid lactate levels. Other clinical features include exacerbated encephalopathy during an infection, hypotonia, microcephaly, epilepsy, and ophthalmoplegia.

Dominant Ataxia Syndromes

1. Spinocerebellar Ataxia-28

[0133] Spinocerebellar ataxia-28 (SCA28) is an autosomal dominant disorder caused by heterozygous mutation in the AFG3L2 gene. The AFG3L2 gene encodes the AFG3-like protein 2 (AFG3L2), an ATP-dependent protease localized to the mitochondrial inner membrane where it forms the catalytic subunit of the m-AAA protease, which degrades misfolded proteins and regulates ribosome assembly. Mutations associated with SCA28 include the following missense mutations: N432T; S674L; E691K; A694E; R702Q; and Y689H. SCA28 is characterized by cerebellar ataxia, dysarthria, nystagmus, ophthalmoparesis, ptosis, slow saccades, hyperreflexia in legs, extensor plantar response, leg and arm spasticity, myoclonic epilepsy, and cerebellar atrophy.

2. Autosomal Dominant Cerebellar Ataxia, Deafness, and Narcolepsy

[0134] Autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN) is caused by heterozygous mutation in the DNMT1 gene. The DNMT1 gene encodes DNA (cytosine-5)-methyltransferases (DNMTs), such as DNMT1, which maintain patterns of methylated cytosine residues in the mammalian genome. Methylation patterns are responsible for the repression of parasitic sequence elements and the expression status of genes subject to genomic imprinting and X inactivation. Faithful maintenance of methylation patterns is required for normal mammalian development, and aberrant methylation patterns are associated with certain human tumors and developmental abnormalities. Mutations of DNMT1 associated with the development of ADCA-DN include the following missense mutations: Ala570Val; Cys596Arg; and Val606Phe.

[0135] ADCA-DN is characterized by adult onset of progressive cerebellar ataxia, narcolepsy/cataplexy, sensorineural deafness, and dementia. More variable features include optic atrophy, sensory neuropathy, psychosis, and depression. Increased lipid levels are observed on muscle biopsy.

3. Optic Atrophy-1

[0136] Optic atrophy-1 (OPA1) is an autosomal dominant optic atrophy caused by heterozygous mutation in the OPA1 gene encoding a dynamin-like 120 kDa GTPase that localizes to the inner mitochondrial membrane where it regulates cellular processes including the stability of the mitochondrial network, mitochondrial bioenergetics output, and the

sequestration of pro-apoptotic cytochrome c oxidase molecules within the mitochondrial cristae spaces. OPA1 directly interacts with subunits of complexes I, II, and III, and an apoptosis inducing factor. Over 100 different OPA1 mutations have been identified, most of which are localized in the GTPase domain of the OPA1 protein. OPA1 mutations can cause oxidative phosphorylation defects at the level of complex I, impairment in mitochondrial ATP synthesis driven by complex I substrates, fibroblasts which are more prone to death, and abnormal mitochondrial morphology.

[0137] OPA1 is characterized by an insidious onset of visual impairment in early childhood with moderate to severe loss of visual acuity, temporal optic disc pallor, color vision deficits, and centrocecal scotoma of variable density. Some patients with mutations in the OPA1 gene may also develop extraocular neurologic features, such as deafness, progressive external ophthalmoplegia, muscle cramps, hyperreflexia, and ataxia.

Variable Ataxia Syndromes

1. CAPOS Syndrome

[0138] Cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) syndrome is a neurologic disorder associated with heterozygous mutation in the ATP1A3 gene. The ATP1A3 gene encodes a Na+/K+ ATPase subunit α3, which forms a catalytic component of the active enzyme that catalyzes the hydrolysis of ATP coupled with the exchange of sodium and potassium ions across the plasma membrane.

[0139] CAPOS is characterized by early-childhood onset of recurrent episodes of acute ataxic encephalopathy associated with febrile illnesses. These acute episodes tend to decrease with time, but the neurologic sequelae are permanent and progressive, resulting in gait and limb ataxia and areflexia. Affected individuals also develop progressive visual impairment due to optic atrophy and sensorineural hearing loss beginning in childhood. More variable features include abnormal eye movements, pes cavus, and dysphagia.

2. Spinocerebellar Ataxia 7

[0140] Spinocerebellar ataxia 7 (SCA7) is caused by an expanded trinucleotide repeat in the gene encoding ataxin-7 (ATXN7). The ATXN7 gene encodes ataxin 7, a transcription factor that appears to be critically important for chromatin remodeling at the level of histone acetylation and deubiquitination. SCA7 is a neurodegenerative disorder characterized by

adult onset of progressive ataxia associated with pigmental macular dystrophy. Associated neurologic signs, such as ophthalmoplegia, pyramidal or extrapyramidal signs, deep sensory loss, or dementia, are also variable.

Barth Syndrome

[0141] Barth Syndrome is a heritable disorder of phospholipid metabolism characterized by dilated cardiomyopathy (DCM), skeletal myopathy, neutropenia, growth delay and organic aciduria. The prevalence of Barth Syndrome is estimated at 1/454,000 live births, with an estimated incidence ranging from 1/400,000 to 1/140,000 depending on geographic location. Barth Syndrome is an X-linked disorder, and so disproportionately affects male patients.

[0142] Barth Syndrome is caused by mutations in the TAZ gene (tafazzin). Defective TAZ1 function results in abnormal remodeling of cardiolipin and compromises mitochondrial structure and respiratory chain function. TAZ1 is expressed at high levels in cardiac and skeletal muscle and is involved in the maintenance of the inner membrane of mitochondria. TAZ1 is involved in maintaining levels of cardiolipin, which is essential for energy production in the mitochondria.

[0143] Clinical presentation of Barth Syndrome is highly variable. Most subjects develop DCM during the first decade of life, and typically during the first year of life, which may be accompanied by endocardial fibroelastosis (EFE) and/or left ventricular noncompaction (LVNC). The manifestations of Barth Syndrome may begin in utero, causing cardiac failure, fetal hydrops and miscarriage or stillbirth during the 2nd/3rd trimester of pregnancy. Ventricular arrhythmia, especially during adolescence, can lead to sudden cardiac death. There is a significant risk of stroke. Skeletal (mostly proximal) myopathy causes delayed motor milestones, hypotonia, severe lethargy or exercise intolerance. There is a tendency to hypoglycemia during the neonatal period. Ninety percent of patients show mild to severe intermittent or persistent neutropenia with a risk of septicemia, severe bacterial sepsis, mouth ulcers and painful gums. Lactic acidosis and mild anemia may occur. Affected boys usually show delayed puberty and growth delay that is observed until the late teens or early twenties, when a substantial growth spurt often occurs. Patients may also present severe difficulties with adequate food intake. Episodic diarrhea is common. Many patients have a similar facial appearance with chubby cheeks, deep-set eyes and prominent ears. Skewed X chromosome inactivation is common in female carriers.

Biotinidase Deficiency

[0144] Biotinidase deficiency is an autosomal recessive metabolic disorder associated with mutations in the BTD gene. In biotinidase deficiency, biotin is not released from proteins in the diet during digestion or from normal protein turnover in the cell. Biotinidase deficiency is associated with mutations in the BTD gene. Biotin, also called vitamin B7, is an important water-soluble nutrient that aids in the metabolism of fats, carbohydrates, and proteins. Biotin deficiency can result in behavioral disorders, lack of coordination, learning disabilities and seizures. Biotin supplementation can alleviate and sometimes totally arrest such symptoms

Blindness

1. Gyrate Atrophy

[0145] Gyrate atrophy of the choroid and retina is caused by a homozygous or compound heterozygous mutation in the OAT gene and is usually observed in Finnish families. Gyrate atrophy of the choroid and retina due to deficiency of ornithine aminotransferase typically begins in late childhood and is clinically characterized by a triad of progressive chorioretinal degeneration, early cataract formation, and type II muscle fiber atrophy. Characteristic chorioretinal atrophy with progressive constriction of the visual fields leads to blindness at the latest during the sixth decade of life. Clinical symptoms include night-blindness, weakness, glutei atrophy, scapular winging, type 2 muscular atrophy, tubular aggregation, mental retardation, hyperornithinemia, and white matter lesions.

2. Dominant Optic Atrophy

[0146] Dominant optic atrophy (DOA), also known as Kjer's optic neuropathy, is an autosomally inherited neuro-ophthalmic disease characterized by a bilateral degeneration of the optic nerves, causing insidious visual loss, typically starting during the first decade of life. The disease affects primarily the retinal ganglion cells (RGC) and their axons forming the optic nerve, which transfer the visual information from the photoreceptors to the lateral geniculus in the brain. Vision loss in DOA is due to optic nerve fiber loss from mitochondria dysfunction. DOA patients usually suffer of moderate visual loss, associated with central or paracentral visual field deficits and color vision defects. The severity of the disease is highly variable, the visual acuity ranging from normal to legal blindness. An ophthalmic examination of a subject with DOA presents isolated optic disc pallor or atrophy, related to the RGC death. About 20% of DOA patients harbor extraocular multi-systemic features,

including neurosensory hearing loss, or less commonly chronic progressive external ophthalmoplegia, myopathy, peripheral neuropathy, multiple sclerosis-like illness, spastic paraplegia or cataracts.

[0147] Two genes (OPA1, OPA3), which encode inner mitochondrial membrane proteins, and three loci (OPA4, OPA5, OPA8) are known to cause DOA. All OPA genes identified encode mitochondrial proteins embedded in the inner membrane and are ubiquitously expressed. OPA1 mutations affect mitochondrial fusion, energy metabolism, control of apoptosis, calcium clearance and maintenance of mitochondrial genome integrity. OPA3 mutations only affect the energy metabolism and the control of apoptosis. OPA1 is the major gene responsible for DOA.

[0148] In most cases, DOA presents as a non-syndromic, bilateral optic neuropathy. Although DOA is usually diagnosed in school-aged children complaining of reading problems, the condition can manifest later, during adult life. On fundus examination, the optic disk typically presents a bilateral and symmetrical pallor of its temporal side with the loss of RGC fibers entering the optic nerve. The optic nerve rim is atrophic and a temporal grey crescent is often present. Optic disc excavation may also be present. Optical Coherence Tomography (OCT) discloses the reduction of the thickness of the peripapillary retinal nerve fiber layer in all four quadrants, but does not disclose alteration of other retinal layers. The visual field typically shows a cecocentral scotoma, and less frequently a central or paracentral scotoma, while peripheral visual field remains normal. Importantly, there is a specific tritanopia, *i.e.*, a blue-yellow axis of color confusion, which, when found, is strongly indicative of DOA. The pupillary reflex and circadian rhythms are not affected, suggesting that the melanopsin RGC are spared during the course of the disease.

[0149] In Syndromic Dominant Optic Atrophy and Deafness (Syndromic DOAD) and Dominant Optic Atrophy *plus* (DOA*plus*) patients experience full penetrance and usually more severe visual deficits. DOAD and DOA*plus* with extra-ophthalmological abnormalities represent up to 20% of DOA patients with an OPA1 mutation. The most common extra-ocular sign in DOA is sensorineural hearing loss, but other associated findings may occur later during life (*e.g.,* myopathy and peripheral neuropathy), suggesting that there is a continuum of clinical presentations ranging from a mild "pure DOA" affecting only the optic nerve to a severe and multi-systemic presentations. Sensorineural hearing loss associated to DOA may range from severe and congenital to subclinical with intra- and inter- familial

variations, and mostly segregate with the OPA1 R445H (c.1334G>A) mutation. In general, auditory brain stem responses, which reflect the integrity of the auditory pathway from the auditory nerve to the inferior colliculus, are absent, but both ears show normal evoked otoacoustic emissions, reflecting the functionality of presynaptic elements and in particular that of the outer hair cells.

3. LHON

[0150] Leber's hereditary optic neuropathy (LHON) is a maternally inherited blinding disease with variable penetrance. LHON is usually due to one of three pathogenic mitochondrial DNA (mtDNA) point mutations. These mutations are at nucleotide positions 11778 G to A, 3460 G to A and 14484 T to C, respectively in the MTND4, MTND1 and MTND6 subunit genes of complex I of the oxidative phosphorylation chain in mitochondria. Reduced efficiency of ATP synthesis and increased oxidative stress are believed to sensitize the retinal ganglion cells to apoptosis.

[0151] Leber's hereditary optic neuropathy (LHON) is characterized by severe visual loss, which usually does not manifest until young adulthood. Maternal transmission is due to a mitochondrial DNA (mtDNA) mutation affecting nucleotide positions (nps) 11778/ND4, 14484/ ND6, or 3460/ND1. These three mutations, affecting respiratory complex I, account for about 95% of LHON cases. Patients inherit multicopy mtDNA entirely from the mother (via the oocyte). The mitochondria may carry only wild-type or only LHON mutant mtDNA (homoplasmy), or a mixture of mutant and wild-type mtDNA (heteroplasmy). Only high loads of mutant heteroplasmy or, most frequently, homoplasmic mutant mtDNA in the target tissue put the subject at risk for blindness from LHON. Except for patients carrying the 14484/ND6 mutation (who present with a more benign disease course), most patients remain legally blind. Typically, a subject in his second or third decade of life will present with abrupt and profound loss of vision in one eye, followed weeks to months later by similar loss of vision in the other eye. LHON may occur later in life and affects both men and women. Environmental factors may trigger the visual loss but do not fully explain why only certain individuals within a family become symptomatic. Additional symptoms include disc microangiopathy, pseudo disc edema, vascular tortuosity, optic atrophy, cardiac conduction defects, spastic paraparesis, sexual and urinary disturbances, Sudden Infant Death Syndrome, abnormal visual evoked potentials, spastic dystonia and encephalopathy.

[0152] Disruptions in ND4 can also lead to spastic paraparesis which is associated with leg stiffness, abnormal visual evoked potentials and sexual and urinary disturbances.

4. Wolfram Syndrome 1

[0153] Wolfram Syndrome-1 (WFS1) is a rare and severe autosomal recessive neurodegenerative disease caused by homozygous or compound heterozygous mutations in the wolframin gene. Wolframin encodes an endoglycosidase H-sensitive glycoprotein that is expressed in the heart, brain, pancreas, liver, kidney, skeletal muscle, hippocampus CA1, amygdaloid areas, olfactory tubercle and allocortex. WFS1 is characterized by diabetes mellitus, optic atrophy, diabetes insipidus, and deafness (DIDMOAD). Additional clinical features may include renal abnormalities, ataxia, Nystagmus, polyneuropathy, central respiratory failure, myoclonus, seizures, organic brain syndrome, dementia or mental retardation, urinary tract atony, orthostatic hypotension, gastroparesis, and diverse psychiatric illnesses. The minimal diagnostic criteria for Wolfram Syndrome are optic atrophy and diabetes mellitus of juvenile onset. Hearing impairment in Wolfram Syndrome is typically progressive and mainly affects the higher frequencies, but a small fraction of affected individuals have congenital deafness.

[0154] Autosomal dominant mutations in the WFS1 gene have been found to cause low-frequency nonsyndromic deafness as well as a Wolfram Syndrome-like phenotype in which affected individuals have hearing impairment with diabetes mellitus and/or optic atrophy.

[0155] Some cases of Wolfram Syndrome of early-onset diabetes mellitus, optic atrophy, and deafness may have their basis in a mitochondrial mutation, such as a 7.6-kb heteroplasmic deletion of mtDNA extending from nucleotide 6466 to nucleotide 14134, inclusive. Some patients exhibit mild hyperlactatemia or morphologic and biochemical abnormalities of the mitochondria. A high percentage of DIDMOAD patients harbor so-called secondary LHON mutations, and both DIDMOAD and LHON patients are concentrated in 2 different mitochondrial haplotypes defined by sets of polymorphisms in ND and tRNA genes. Thus, the different clinical features of the mitochondrial disease groups investigated correspond to different clusters of mtDNA variants, which might act as predisposing haplotypes, increasing the risk for the given disease.

5. Wolfram Syndrome 2

[0156] Wolfram Syndrome-2 (WFS2) is an autosomal recessive neurodegenerative disorder caused by homozygous mutations in the CISD2 (ERIS) gene, which encodes CDGSH iron sulfur domain protein 2. CISD2 is an endoplasmic reticulum-localized zinc finger protein that is expressed in pancreas, brain and other tissues. WFS2 is characterized by diabetes mellitus, mild diabetes insipidus, high frequency sensorineural hearing loss, optic atrophy or neuropathy, urinary tract dilatation, and defective platelet aggregation resulting in peptic ulcer bleeding.

6. Age-related Macular Degeneration

[0157] Age-related macular degeneration (ARMD) is a common complex disorder that affects the central region of the retina and is the leading cause of blindness in Caucasian Americans over 65 years of age. Susceptibility to ARMD is associated with mutations in the ARMS2 gene, which encodes a deduced 107-amino acid protein with nine predicted phosphorylation sites and a molecular mass of 12 kD. The ARMS2 protein is localized to the mitochondrial outer membrane.

Brunner Syndrome

[0158] Brunner Syndrome is a form of X-linked non-dysmorphic mild mental retardation. Two monoamine oxidase isoenzymes, monoamine oxidase A (MAOA) and monoamine oxidase B (MAOB), are closely linked in opposite orientation on the X chromosome and are expressed in the outer mitochondrial membrane. MAOA and MAOB oxidize neurotransmitters and dietary amines, the regulation of which is important to the maintenance of normal mental states. MAOA prefers the monoamines serotonin, norepinephrine, and dopamine as substrates, while MAOB prefers phenylethylamine. Brunner Syndrome is caused by an MAOA deficiency, which results in an accumulation of serotonin, dopamine, and epinephrine, in the brain. Mutations in the MAOA gene have been associated with aggressive, violent, impulsive, autistic, and antisocial behaviors.

Cardiomyopathy

1. Left Ventricular Noncompaction

[0159] Left ventricular noncompaction-1 (LVNC1) is caused by a heterozygous mutation in the alpha-dystrobrevin gene and is characterized by numerous prominent trabeculations and deep intertrabecular recesses in hypertrophied and hypokinetic segments of the left ventricle.

[0160] The developing myocardium gradually condenses, and the large spaces within the trabecular meshwork flatten or disappear. Isolated noncompaction of ventricular myocardium, sometimes called spongy myocardium or persisting myocardial sinusoids, represents an arrest in endomyocardial morphogenesis, and is characterized by numerous, excessively prominent trabeculations and deep intertrabecular recesses. LVNC may occur in isolation or in association with congenital heart disease. Distinctive morphologic features can be recognized on 2-dimensional echocardiography. Clinical manifestations of the disorder included depressed left ventricular systolic function, ventricular arrhythmias, systemic embolization, and distinctive facial dysmorphism.

[0161] The clinical presentation of left ventricular noncompaction is highly variable, ranging from asymptomatic to severe heart failure and sudden death. Higher occurrence of familial cases, facial dysmorphism, and congenital arrhythmias such as Wolff-Parkinson-White Syndrome are observed in children, whereas secondary arrhythmias, such as atrial fibrillation, are more common in adults. The mode of inheritance is predominantly autosomal dominant, and sarcomere protein mutations are more common in adults.

2. Infantile Histiocytoid Cardiomyopathy

[0162] Histiocytoid cardiomyopathy goes by various names, including infantile xanthomatous cardiomyopathy, focal lipid cardiomyopathy, oncocytic cardiomyopathy, infantile cardiomyopathy with histiocytoid change, and foamy myocardial transformation of infancy. The disorder is caused by mutations in the gene encoding mitochondrial cytochrome b (MTCYB) and is a rare but distinctive entity of infancy and childhood characterized by the presence of characteristic pale granular foamy histiocyte-like cells within the myocardium. It usually affects children younger than 2 years of age, with a clear predominance of females over males. Infants present with dysrhythmia or cardiac arrest, and the clinical course is usually fulminant, sometimes simulating Sudden Infant Death Syndrome. Clinical features include high frequency of anomalies involving the nervous system and eyes and of oncocytic cells in various glands. Because of the large number of mitochondria present in the histiocytoid cells, they resemble oncocytes. Other disorders involving MTCYB mutations include exercise intolerance, myopathy, LHON, Familial Myalgia Syndrome, colorectal cancer, encephalomyopathy, Parkinsonism, and susceptibility to obesity.

3. Cardioencephalomyopathy with Cytochrome c Oxidase Deficiency (CEMCOX1)

[0163] Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency-1 (CEMCOX1) can be caused by a compound heterozygous mutation in the SCO2 gene, a COX assembly gene on chromosome 22q13. Another form of fatal infantile cardioencephalomyopathy due to COX deficiency, CEMCOX2 is caused by mutations in the COX15 gene on chromosome 10q24. Age of onset for infantile cardioencephalomyopathy is usually within the first 3 months of infancy and is equally prevalent between males and females. Infants usually die at 6 months.

[0164] Infants with COX deficiency caused by a mutation in the SCO2 gene present with a fatal infantile cardioencephalomyopathy characterized by hypertrophic cardiomyopathy, lactic acidosis, and gliosis. Heart and skeletal muscle show reductions in COX activity, whereas liver and fibroblasts show mild COX deficiencies. Patients show a severe reduction of the mitochondrial-encoded COX I and II subunits, whereas the nuclear-encoded COX subunits IV and Va are present but reduced in intensity. Clinical features include hypotonia, limb spasticity, muscle atrophy or denervation, respiratory difficulties, increased blood and CSF lactate, hypertrophic cardiomyopathy, seizures, psychomotor retardation, Leigh-like Syndrome, neutropenia, ptosis, gliosis, ophthalmoplegia, encephalopathy, and severely reduced COX activity.

4. Sengers Syndrome: Cardiomyopathy, Hypertrophic & Cataracts

[0165] Sengers Syndrome, also known as cardiomyopathic mitochondrial DNA depletion syndrome-10 (MTDPS10), is caused by a homozygous or compound heterozygous mutation in the AGK gene. Sengers Syndrome is an autosomal recessive mitochondrial disorder characterized by congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy, exercise intolerance, and lactic acidosis. Mental development is normal, but affected individuals may die early from cardiomyopathy. Skeletal muscle biopsies of affected individuals show severe mtDNA depletion.

[0166] While several pieces of evidence pointed indirectly to the involvement of oxygen free radicals in the etiology of cardiomyopathy with cataracts, direct evidence showed that complex I deficiency is associated with an excessive production of hydroxyl radicals and lipid peroxidation. Patients with isolated NADH:ubiquinone oxidoreductase deficiency (or complex I deficiency) most commonly present with fatal neonatal lactic acidosis or with

Leigh disease. Although the clinical features of Sengers Syndrome suggest a mitochondrial disorder, no abnormalities are found on routine mitochondrial biochemical diagnostics, viz., the determination of pyruvate oxidation rates and enzyme measurements. Protein content of mitochondrial ANT1 is strongly reduced in the muscle tissues of affected patients with Sengers Syndrome. Additional clinical phenotypes for cardiomyopathy with cataracts include hepatopathy, tubulopathy, hypotonia, and mild developmental delay.

5. Cardiofaciocutaneous Syndrome 1 (CFC1)

[0167] Cardiofaciocutaneous Syndrome-1 (CFC1), caused by disruptions in the BRAF gene, is a multiple congenital anomaly disorder characterized by a distinctive facial appearance, heart defects, and mental retardation. The heart defects include pulmonic stenosis, atrial septal defect, and hypertrophic cardiomyopathy. Some patients have ectodermal abnormalities such as sparse and friable hair, hyperkeratotic skin lesions, and a generalized ichthyosis-like condition. Typical facial characteristics include high forehead with bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge, and posteriorly angulated ears with prominent helices. Most cases occur sporadically, but autosomal dominant transmission has been rarely reported.

[0168] Disruptions in BRAF gene function may impact the TCA cycle and oxidative metabolism. Clinical features include atopic dermatitis, ichthyosis, hyperkeratosis (extensor surfaces), keratosis pilaris, multiple palmar creases, multiple lentigines, sparse hair growth, optic nerve dysplasia, increased tendon reflexes, extensor plantar response, increased sensitivity to light touch, mental retardation, seizures, cortical atrophy, hypoplasia, ptosis, strabismus, oculomotor apraxia, nystagmus, hypertelorism, exophthalmos, prominent philtrum, micrognathia, macrocephaly, joint hyperextensibility, osteopenia, clinodactyly, pectus excavatum or carinatum, short stature, GI dysmotility, splenomegaly, enlarged mitochondria, increased frequency of 2C fibers, reduced CoQ10, ventriculomegaly, hypsarrhythmia; and focal epileptiform discharges.

6. Trifunctional Protein Deficiency

[0169] Mitochondrial trifunctional protein (MTP) deficiency can be caused by mutations in the genes encoding either the alpha (HADHA) or beta (HADHB) subunits of the mitochondrial trifunctional protein. The mitochondrial trifunctional protein, composed of 4 alpha and 4 beta subunits, catalyzes 3 steps in mitochondrial beta-oxidation of fatty acids:

long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), long-chain enoyl-CoA hydratase, and long-chain thiolase activities. Trifunctional protein deficiency is characterized by decreased activity of all 3 enzymes. Clinically, classic trifunctional protein deficiency can be classified into 3 main clinical phenotypes: neonatal onset of a severe, lethal condition resulting in sudden unexplained infant death (SIDS), infantile onset of a hepatic Reye-like syndrome, and late-adolescent onset of primarily a skeletal myopathy.

[0170] Some patients with MTP deficiency show a protracted progressive course associated with myopathy, recurrent rhabdomyolysis, and sensorimotor axonal neuropathy. These patients tend to survive into adolescence and adulthood. Clinical features include cardiomyopathy, myopathy, liver dysfunction, encephalopathy, Sudden Infant Death Syndrome, and axonal neuropathy. Variant syndromes comprising MTP mutations include hepatic with recurrent Hypoketotic hypoglycemia, later-onset axonal sensory neuropathy ± episodic myoglobinuria, and early-onset axonal sensory neuropathy.

7. Encephalocardiomyopathy with Cytochrome c Oxidase Deficiency

[0171] Mutations in the nuclear gene C12ORF62 have been associated with fatal infantile encephalocardiomyopathy with cytochrome c oxidase deficiency. C12ORF62 is a membrane-associated protein that localizes to the mitochondria and promotes COX I assembly & coupling with assembly of nascent subunits into COX holoenzyme complex. Clinical symptoms include reduced COX activity, neonatal lactic acidosis, oligoamnios, septum-lucidum cysts, hypotelorism, microphthalmia, ogival palate, single palmar crease, hypertrophic cardiomyopathy, hepatomegaly, renal hypoplasia, adrenal hyperplasia, brain hypertrophy, white-matter myelination, and cavities in parieto-occipital region, brainstem, and cerebellum.

8. Cardiomyopathy + Encephalopathy

[0172] Mutations in NADH-Ubiquinone oxidoreductase Fe-S Protein 2 (NDUFS2) have been associated with cardiomyopathy + encephalomyopathy. NDUFS2 is a component of complex I and plays a role in protein transport. The age of onset for the disorder is from birth to 7 months. Infants usually die before 2 to 3 years of age. Clinical symptoms include axial hypotonia, failure to thrive, hypertrophic cardiomyopathy, limb hyperreflexia, optic atrophy, nystagmus, progressive encephalopathy, sleep apnea, high lactate levels in CSF & blood, hypodensities in basal ganglia; generalized brain atrophy, and reduced complex I activity.

9. Mitochondrial Phosphate Carrier Deficiency

[0173] Mitochondrial phosphate carrier deficiency can be caused by disruptions in SLC25A3, which encodes a mitochondrial solute carrier. SLC25A3 aids in the import of inorganic phosphate into mitochondrial matrix and functions in ATP synthesis. Mitochondrial phosphate carrier deficiency is characterized by progressive hypertrophic cardiomyopathy, respiratory failure, hypotonia, high serum lactate, lipid accumulation in type I muscle fibers, abnormal mitochondrial network, defective ATP synthesis and reduced mitochondrial phosphate carrier activity. Some infants die in the 1st year, whereas those that survive to adulthood exhibit exercise intolerance and proximal weakness.

10. Fatal infantile Cardioencephalomyopathy, due to Cytochrome c Oxidase Deficiency 2 (CEMCOX2)

[0174] Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency (CEMCOX2) can be caused by a compound heterozygous mutation in the COX15 gene. Shortly after birth, patients present with seizures, hypotonia, and lactic acidosis. Other clinical symptoms include midface hypoplasia, biventricular hypertrophic cardiomyopathy and reduced Complex IV activity. Patients usually die within 1 month of life.

11. Leigh Syndrome

[0175] Leigh Syndrome mutations have been identified in both nuclear- and mitochondrial-encoded genes involved in energy metabolism, including mitochondrial respiratory chain complexes I, II, III, IV, and V, which are involved in oxidative phosphorylation and the generation of ATP, and components of the pyruvate dehydrogenase complex.

[0176] Mutations in complex I genes include mitochondrial-encoded MTND2, MTND3, MTND5, and MTND6, the nuclear-encoded NDUFS1, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFA2, NDUFA9, NDUFA10, NDUFA12, NDUFAF6, FOXRED1, COXPD15 and C20ORF7, and the complex I assembly factor NDUFAF2. A mutation in the MTFMT gene, which is involved in mitochondrial translation, has also been reported with complex I deficiency.

[0177] Leigh Syndrome is also associated with mutations in complex I (C8ORF38), complex II (the flavoprotein subunit A (SDHA)); complex III (BCS1L); complex IV (MTCO3, COX10, COX15, SCO2, SURF1, TACO1, and PET100); complex V (MTATP6);

mitochondrial tRNA proteins (MTTV, MTTS2, MTTK, MTTW, and MTTL1); components of the pyruvate dehydrogenase complex (*e.g.*, DLD and PDHA1); the LRPPRC gene; and coenzyme Q10.

[0178] Leigh Syndrome is an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral lesions in one or more areas of the central nervous system, including the brainstem, thalamus, basal ganglia, cerebellum, and spinal cord. Age of onset is around 1 year and patients usually die within 2 years of onset. The lesions are areas of demyelination, gliosis, necrosis, spongiosis, or capillary proliferation. Clinical features also include hypotonia, ataxia, vomiting, choreoathetosis, hyperventilation, encephalopathy, loss verbal milestones, motor spasticity, abnormal breathing rhythm, hearing loss, nystagmus, dystonia, visual loss, ophthalmoparesis, optic atrophy, peripheral neuropathy, intercurrent infection, cog-wheel rigidity, distal renal tubular acidosis, limb athetosis, seizures, carbohydrate intolerance, COX deficiency in muscle, lactic acidosis with hypoglycemia, kyphoscoliosis, short stature, brisk tendon reflexes, obesity, and high lactate levels in CSF. Clinical symptoms depend on which areas of the central nervous system are involved. The most common underlying cause is a defect in oxidative phosphorylation.

[0179] Mutations in NDUFV2, MTND2, MTND5, and MTND6 can result in Leigh Syndrome due to mitochondrial complex I deficiency. Clinical symptoms include reduced Complex I activity, hypertrophic cardiomyopathy, developmental delay, cerebral atrophy, hypoplasia of the corpus callosum, acidosis, seizures, coma, cardiovascular arrest, demyelinization of corticospinal tracts, subacute necrotizing encephalomyelopathy, progressive encephalopathy, respiratory failure, exercise intolerance, weakness, mitochondrial proliferation in muscle, motor retardation, hypotonia, deafness, dystonia, pyramidal features, brainstem events with oculomotor palsies, strabismus & recurrent apnea, lactic acidemia, and basal ganglia lesions.

[0180] Mutations in MTCO3 can result in Leigh Syndrome that usually presents at 4 years of age. Clinical symptoms include spastic paraparesis with ophthalmoplegia, high serum lactic acid, Leigh-like lesions in putamen, and reduced COX activity in muscle. Mutations also found in MTCO3 cause LHON, Myopathy with exercise intolerance, rhabdomyolysis, episodic encephalopathy, and nonarteritic ischemic optic neuropathy (NAION)-Myoclonic epilepsy.

[0181] Mutations in 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) results in β-Hydroxyisobutyryl CoA Deacylase (HIBCH) deficiency, a Leigh-like Syndrome that usually manifests at neonatal to 6 months of age. Symptoms include hypotonia, regression, poor feeding, dystonia, ataxia, seizures, dysmorphic facies, vertebral anomalies, tetralogy of fallot, progressive or acute encephalopathy, respiratory chain deficiencies, high CSF lactate, basal ganglia abnormalities, brain agenesis and accumulation of metabolites (*e.g.*, methacrylyl-CoA, acryloyl-CoA, hydroxy-C4-carnitine).

[0182] Mutations in enoyl-CoA hydratase, short-chain, 1 (ECHS1) results in Short chain enoyl-CoA hydratase (ECHS1) deficiency, a Leigh-like Syndrome that presents at the neonatal stage. ECHS1 catalyzes the second step in mitochondrial fatty acid β-oxidation. Clinical symptoms include hypotonia, respiratory insufficiency or apnea, bradycardia, developmental delay, high serum lactate, white matter atrophy, and accumulation of metabolites (*e.g.*, methacrylyl-CoA, acryloyl-CoA).

[0183] Mutations in ATP synthase 6 (MTATP6) result in Maternal Inheritance Leigh Syndrome (MILS). Clinical symptoms include hypotonia, developmental delay, peripheral neuropathy, seizures, retinitis pigmentosa or optic atrophy, ataxia, respiratory failure, bilateral striatal necrosis, hereditary spastic paraparesis, myelopathy, limb spasticity, weakness, and sensory loss or pain.

12. Dilated Cardiomyopathy with Ataxia (DCMA)

[0184] 3-methylglutaconic aciduria type V (MGCA5), also called dilated cardiomyopathy with ataxia (DCMA), is caused by a homozygous mutation in the DNAJC19 gene on chromosome 3q26. DNAJC19 encodes a DNAJ domain-containing protein that is localized to the inner mitochondrial membrane (TIM) and may be involved in molecular chaperone systems of Hsp70/Hsp40 type. Mitochondrial import inner membrane translocase subunit TIM14 may also act as a co-chaperone that stimulates the ATP-dependent activity.

[0185] DCMA is an autosomal recessive disorder characterized by the onset of dilated or noncompaction cardiomyopathy in infancy or early childhood. Clinical symptoms include cardiomyopathy, long Q-T syndrome, cerebellar ataxia, delayed psychomotor development, optic atrophy, mental retardation, seizures, testicular dysgenesis, cryptorchidism to severe perineal hypospadias, growth retardation, microcytic anemia, mild muscle weakness, mildly elevated hepatic enzymes and increased urinary excretion of 3-methylglutaconic acid.

13. Mitochondrial DNA Depletion Syndrome 12 (Cardiomyopathic type)

[0186] Mitochondrial DNA Depletion Syndrome-12 (MTDPS12) is caused by homozygous mutations in the Adenine nucleotide translocator 1 (ANT1) gene. Heterozygous mutations in the ANT1 gene cause autosomal dominant progressive external ophthalmoplegia-2 (PEOA2).

[0187] Mitochondrial DNA Depletion Syndrome-12 is an autosomal recessive mitochondrial disorder characterized by childhood onset of slowly progressive hypertrophic cardiomyopathy and generalized skeletal myopathy resulting in exercise intolerance and, in some patients, muscle weakness, pain and atrophy. Skeletal muscle biopsy shows ragged red fibers, mtDNA depletion, and accumulation of abnormal mitochondria. Clinical symptoms include headache episodes, nausea, vomiting, moderately high levels of creatine kinase in serum, ankle contractures, lactic acidosis, hyperalaninemia, SDH-positive muscle fibers, multiple mtDNA deletions or depletions, abnormal mitochondria containing paracrystalline inclusions, high citrate synthase levels, partial reductions in complexes I, III, IV and V, cardiomyocyte degeneration, subendocardial interstitial fibrosis, and arteriolar smooth muscle hypertrophy.

14. Cardiomyopathy due to Mitochondrial tRNA Deficiencies

[0188] Mutations in mtRNA Ile (MTTI) can result in fatal infantile onset cardiomyopathy. Clinical features of other mtRNA Ile syndromes include cardiac dilation and hypertrophy, short stature, deafness, some MELAS symptoms, death due to cardiac failure, low mitochondrial oxidative enzymes, familial progressive necrotizing encephalopathy, impaired glucose tolerance, hyperlipidemia, hyperuricemia, progressive myoclonus epilepsy, ragged red fibers, spastic paraparesis, ataxia; PEO; mental retardation, diabetes mellitus, hypomagnesemia, hypokalemia, hypertension, hypercholesterolemia, increased prevalence of migraine headache, and rhabdomyolysis.

[0189] Mutations in mtRNA Lys can result in neonatal hypertrophic cardiomyopathy, diabetes, MERRF, high lactate and pyruvate levels in serum and infantile death.

[0190] Mutations in mtRNA Leu (MTTL1) can result in hypertrophic cardiomyopathy Barth-like Syndrome, diabetes, dilated cardiomyopathy, MERRF/KSS, MELAS, MERRF-like with diabetes, optic neuropathy & retinopathy, left ventricular noncompaction, Wolff-Parkinson-White conduction defects, Sudden Infant death Syndrome, riboflavin sensitive

myopathy, rhabdomyolysis, fatigue, PEO, and the like. Age of onset typically occurs between 24 to 40 years.

[0191] Mutations in mtRNA Leu (MTTL2) can result in adult onset cardiomyopathy, myopathy, sideroblastic anemia, PEO, and encephalomyopathy.

[0192] Mutations in mtRNA His can result in adult onset dilated or hypertrophic cardiomyopathy, pigmentary retinopathy and sensorineural deafness.

[0193] Mutations in mtRNA Gly can result in exercise intolerance, nonobstructive hypertrophic cardiomyopathy (onset age: neonatal to childhood), Complex IV & Complexes II + III deficiencies and sudden death.

15. Cardiomyopathy: Mitochondrial ATP synthase (Complex V) Defects

[0194] Mutations in ATP synthase F1 complex assembly factor-2 (ATP 12), which is required for Complex V biogenesis, can result in mitochondrial complex V (ATP synthase) deficiency nuclear type 1 (MC5DN1). The disorder manifests at birth and subjects exhibit low APGAR scores and birth weight. Biochemically, the patients show a generalized decrease in the content of ATP synthase complex which is less than 30% of normal as well as high plasma lactate levels. Most cases present with neonatal-onset hypotonia, lactic acidosis, hepatomegaly, hyperammonemia, hypertrophic cardiomyopathy, facial dysmorphism, microcephaly, psychomotor and mental retardation, and 3-methylglutaconic aciduria. Many patients die within a few months or years.

[0195] Mutations in mitochondrial ATP synthase 8 (MT-ATP8), a component of Complex V, can also result in apical hypertrophic cardiomyopathy and neuropathy. The disorder manifests during infancy and is associated with delayed motor development, gait and balance disorder, dysarthric speech, extensor plantar response, reduced tendon response, mild external ophthalmoplegia, exercise intolerance, shortness of breath (dyspnea) during exercise, angina and apical left ventricular hypertrophy, neuropathy, reduced Complex V activity & assembly, high lactate levels in CSF and abnormal nerve conduction velocity in legs.

16. Mitochondrial Complex IV Deficiency

[0196] Complex IV (cytochrome c oxidase) is the terminal enzyme of the respiratory chain and consists of 13 polypeptide subunits, 3 of which are encoded by mitochondrial DNA. The

3 mitochondrial encoded proteins in the cytochrome oxidase complex are the actual catalytic subunits that carry out the electron transport function.

[0197] Cytochrome c oxidase deficiency can be caused by mutations in several nuclear-encoded and mitochondrial-encoded genes. Mutations associated with the disorder have been identified in several mitochondrial COX genes, MTCO1, MTCO2, MTCO3, as well as in mitochondrial tRNA (ser) (MTTS1) and tRNA (leu) (MTTL1). Mutations in nuclear genes include those in COX10, COX6B1, SCO1, FASTKD2, C2ORF64 (COA5), COA6, C12ORF62 (COX14), COX20, and APOPT1. COX deficiency caused by mutations in SCO2 and COX15 have been found to be associated with fatal infantile cardioencephalomyopathy. Cytochrome c oxidase deficiency associated with Leigh Syndrome may be caused by mutations in the SURF1 gene, COX15 gene, TACO1 gene, or PET100 gene. Cytochrome c oxidase deficiency associated with the French Canadian type of Leigh Syndrome (LSFC) is caused by mutations in the LRPPRC gene. Most isolated COX deficiencies are inherited as autosomal recessive disorders caused by mutations in nuclear-encoded genes; mutations in the mtDNA-encoded COX subunit genes are relatively rare.

[0198] Clinical features associated with the disruption of COA5 function include hypertrophic restrictive cardiomyopathy, accumulation of lipid droplets in muscle, reduced Complex IV activity, and mitochondrial proliferation in muscle. Age of onset occurs in less than 1 month or *in utero*.

[0199] Clinical features associated with the disruption of COA6 function include hypertrophic cardiomyopathy, reduced complexes I & IV in cardiac tissue, and multiple respiratory chain defects. Age of onset occurs in less than 1 year.

17. <u>Combined Oxidative Phosphorylation Deficiency 8 (COXPD8)</u>

[0200] Combined oxidative phosphorylation deficiency-8 (COXPD8) is caused by a homozygous or compound heterozygous mutation in the Alanyl-tRNA Synthetase 2 (AARS2) gene, which encodes an amino acid tRNA synthetase.

[0201] COXPD8 is an autosomal recessive disorder due to dysfunction of the mitochondrial respiratory chain. The main clinical manifestation is a lethal infantile hypertrophic cardiomyopathy, but there may also be subtle skeletal muscle and brain involvement. Biochemical studies show combined respiratory chain complex deficiencies in complexes I,

III, and IV in cardiac muscle, skeletal muscle, and brain. The liver is not affected. Muscle cells are positive for both COX & SDH.

[0202] A variant AARS2 syndrome is progressive leukoencephalopathy with ovarian failure (LKENP), an autosomal recessive neurodegenerative disorder characterized by loss of motor and cognitive skills, usually with onset in young adulthood. Some patients may have a history of delayed motor development or learning difficulties in early childhood. Neurologic decline is severe, usually resulting in gait difficulties, ataxia, spasticity, and cognitive decline and dementia. Most patients lose speech and become wheelchair-bound or bedridden. Brain MRI shows progressive white matter signal abnormalities in the deep white matter. Affected females develop premature ovarian failure. Clinical features of LKENP include COX deficiency, ataxia, cerebellar atrophy, spasticity, cognitive decline, delayed development, ovarian failure during the 3rd to 5th decade, loss of motor skills, speech & cognition by 5th decade, and abnormal cerebral white matter.

18. Combined Oxidative Phosphorylation Deficiency 10 (COXPD10)

[0203] Combined oxidative phosphorylation deficiency-10 (COXPD10) is caused by homozygous or compound heterozygous mutations in the mitochondrial translation optimization 1 homolog (MTO1) gene. MTO1 is a mitochondrial-tRNA modifier that is normally expressed in tissues with high metabolic rate, such as skeletal muscle, liver, and heart. COXPD10 is an autosomal recessive disorder resulting in variable defects of mitochondrial oxidative respiration. Affected individuals present in infancy with hypertrophic cardiomyopathy and lactic acidosis. The severity is variable, but can be fatal in the most severe cases. Additional clinical symptoms include oligohydramnios, hypotonia, psychomotor delay, spasticity, seizures, dystonia, hypoglycemia, metabolic acidosis, high serum lactate and reduced Complex I & IV activity.

[0204] Loss of MTO1 function can also result in encephalomyopathy, which usually manifests at 3 months of age. Symptoms include seizures, infantile spasms, delayed motor & cognitive development, axial hypotonia, chorio-athetoid movements, COX negative muscle fibers, and Complex IV deficiency.

19. Combined Oxidative Phosphorylation Deficiency 16 (COXPD16)

[0205] Combined oxidative phosphorylation deficiency-16 (COXPD16) is caused by homozygous mutations in the MRPL44 gene. MRPL44 encodes a structural subunit of

mitochondrial large (39S) ribosomal subunit and plays role in the assembly and stability of the 39S subunit. Age of onset is usually at 3 to 6 months. Clinical features include hypertrophic cardiomyopathy, steatosis, high levels of liver transaminases, high serum lactate, reduced COX staining in muscle, and reduced Complex I and IV activity.

[0206] Other mitochondrial ribosomal subunit disorders include disruptions in MRPS16 and MRPS22, which encode components of the small ribosomal subunit. Symptoms include early death and severe lactic acidosis. Alternatively, disruptions in MRPL3, which encodes a component of the large ribosomal subunit, results in cardiomyopathy and mental retardation.

20. Combined Oxidative Phosphorylation Deficiency 17 (COXPD17)

[0207] Combined oxidative phosphorylation deficiency-17 (COXPD17) is caused by homozygous or compound heterozygous mutations in the ELAC2 gene, which encodes a zinc phosphodiesterase protein with tRNA processing endonuclease activity. The protein also interacts with PTCD1 and is ubiquitously expressed. Combined oxidative phosphorylation deficiency-17 is an autosomal recessive disorder of mitochondrial dysfunction characterized by onset of severe hypertrophic cardiomyopathy in the first year of life. Other features include hypotonia, poor growth, microcephaly, lactic acidosis, delayed psychomotor development, impaired central hearing, high alanine and glutamine levels, abnormal mitochondrial cristae, reduced Complex I and IV activity and failure to thrive. The disorder may be fatal in early childhood.

21. Combined Oxidative Phosphorylation Deficiency 5 (COXPD5)

[0208] Mutations in MRPS22 can result in combined oxidative phosphorylation deficiency-5 (COXPD5). Patients show reduced activities of mitochondrial respiratory chain complexes I, III, and IV, marked and generalized defect in mitochondrial translation, microcephaly, dilated cardiomyopathy, dysmorphic features, hypotonia, metabolic acidosis, transient seizures, poor growth, lack of development, and spastic tetraplegia.

[0209] Mutations in NDUFA11 can result in mitochondrial complex I deficiency. Symptoms include fatal infantile metabolic acidosis, encephalocardiomyopathy with brain atrophy, no motor development, and hypertrophic cardiomyopathy.

22. Combined Oxidative Phosphorylation Deficiency 9 (COXPD9)

[0210] Combined oxidative phosphorylation deficiency-9 (COXPD9) is caused by compound heterozygous mutations in the MRPL3 gene. Patients present with failure to thrive, poor feeding, hypertrophic cardiomyopathy, hepatomegaly, psychomotor retardation, mental retardation, increased plasma lactate and alanine, abnormal liver enzymes, and decrease in activity of mitochondrial respiratory complexes I, III, IV, and V, with a mild decrease in complex II.

Carnitine Disorders

[0211] Carnitine (beta-hydroxy-gamma-trimethylaminobutyric acid) is an essential cofactor for transport of long chain fatty acids across mitochondrial membranes, permitting beta-oxidation. Carnitine in body fluids is derived from the diet or biosynthesis and is actively transported into muscle. Two biochemically and clinically distinct disorders cause low concentrations of carnitine in skeletal muscle. Systemic carnitine deficiency shows low carnitine in the liver and/or plasma. In muscle carnitine deficiency, lipid storage myopathy occurs with low muscle carnitine but normal liver and serum carnitine.

[0212] The age of onset for carnitine deficiency, myopathic form occurs during childhood – early adulthood. Symptoms include weakness, cardiomyopathy, and congestive heart failure.

1. Carnitine Acetyltransferase Deficiency

[0213] Carnitine acetyltransferase (CRAT) deficiency is an autosomal recessive disorder characterized by ataxia, oculomotor palsy, hypotonia, poor respiration, failure to thrive, and altered consciousness. CRAT functions in the maintenance of normal fatty acid metabolism by catalyzing the transfer of acyl groups from acyl-CoA thioester to carnitine. CRAT controls the ratio of acyl-CoA/CoA in mitochondria, peroxisomes, and endoplasmic reticulum. CRAT deficiency has been shown to be associated with deletions of mitochondrial DNA, mainly the ND4-ND4L region, in muscle. Other regions of the mitochondrial genome also showed deletions of varying size and extent, suggesting multiple deletions of the mitochondrial DNA.

2. Carnitine Palmitoyltransferase I Deficiency

[0214] Carnitine palmitoyltransferase I (CPT I) deficiency is an autosomal recessive metabolic disorder that affects the mitochondrial oxidation of long-chain fatty acids in the

liver and kidneys and is characterized by recurrent episodes of fasting-induced hypoketotic hypoglycemia and an elevated risk of liver failure. During metabolic crisis, blood tests reveal hypoglycemia, elevated levels of plasma carnitine and liver transaminases, and mild hyperammonemia. Urine tests may show unusually low levels of ketones, and medium-chain dicarboxylic aciduria. CPT I deficiency is associated with mutations in the CPT1A gene, which encodes carnitine palmitoyltransferase IA. The carnitine palmitoyltransferase enzyme system comprising CPT I and CPT II, in combination with acyl-CoA synthetase and carnitine/acylcarnitine translocase, provides the mechanism by which long-chain fatty acids are transferred from the cytosol to the mitochondrial matrix. The CPT I isozymes, CPT1A and CPT1B are located in the mitochondrial outer membrane, whereas CPT II is located in the inner mitochondrial membrane.

3. Myopathic Carnitine Deficiency

[0215] Myopathic carnitine deficiency is a progressive autosomal recessive disorder characterized by lipid storage myopathy with low muscle carnitine but normal liver and serum carnitine. Clinical features include symmetric, proximal weakness in the face and tongue, cardiomyopathy, congestive heart failure, moderately elevated serum creatine kinase levels.

4. Primary Systemic Carnitine Deficiency

[0216] Primary systemic carnitine deficiency (CDSP) is an autosomal recessive disorder of fatty acid oxidation caused by a homozygous or compound heterozygous mutation in the SLC22A5 gene. The SLC22A5 gene encodes solute carrier family 22 member 5 protein, which functions as a sodium-ion dependent, high affinity carnitine transporter involved in the active cellular uptake of carnitine. Mutations in the SLC22A5 gene result in a defective carnitine transporter, which is expressed in muscle, heart, kidney, and fibroblasts. This results in impaired fatty acid oxidation in skeletal and heart muscle. In addition, renal wasting of carnitine results in low serum levels and diminished hepatic uptake of carnitine by passive diffusion, which impairs ketogenesis. If diagnosed early, all clinical manifestations of the disorder can be completely reversed by supplementation of carnitine. However, if left untreated, patients will develop lethal heart failure.

5. Carnitine Palmitoyltransferase II Deficiency

[0217] Carnitine palmitoyltransferase II (CPT II) deficiency is an autosomal recessive disorder and is the most common inherited disorder of mitochondrial long-chain fatty acid oxidation and is associated with mutations in the CPT2 gene. The CPT2 gene encodes carnitine palmitoyltransferase 2 an enzyme that is essential for fatty acid oxidation. Over 70 different mutations in the CPT2 gene have been identified. CPT2 mutations lead to a reduction in the activity of carnitine palmitoyltransferase 2. There are three main types of CPT II deficiency: a lethal neonatal form, a severe infantile hepatocardiomuscular form, and a myopathic form.

[0218] The lethal neonatal form becomes apparent soon after birth. Infants with this form of CPT II deficiency develop respiratory failure, seizures, liver failure, cardiomyopathy, and arrhythmia. Affected infants also exhibit hypoketotic hypoglycemia and structurally abnormal brain and kidneys. Infants with the lethal neonatal form of CPT II deficiency typically survive for only a few days to a few months.

[0219] The severe infantile hepatocardiomuscular form of CPT II deficiency affects the liver, heart, and muscles. Signs and symptoms usually appear within the first year of life. This form involves recurring episodes of hypoketotic hypoglycemia, seizures, hepatomegaly, cardiomyopathy, and arrhythmia. Problems related to this form of CPT II deficiency can be triggered by periods of fasting or by illnesses such as viral infections. Individuals with the severe infantile hepatocardiomuscular form of CPT II deficiency are at risk for liver failure, nervous system damage, coma, and sudden death.

[0220] The myopathic form is the least severe type of CPT II deficiency. This form is characterized by recurrent episodes of myalgia and rhabdomyolysis. The destruction of muscle tissue results in myoglobinuria. Myoglobin can also damage the kidneys, in some cases leading to life-threatening kidney failure. Episodes of myalgia and rhabdomyolysis may be triggered by exercise, stress, exposure to extreme temperatures, infections, or fasting. The first episode usually occurs during childhood or adolescence. Most people with the myopathic form of CPT II deficiency have no signs or symptoms of the disorder between episodes.

6. Carnitine-acylcarnitine Translocase Deficiency

[0221] Carnitine-acylcarnitine translocase deficiency (CACTD) is an autosomal recessive metabolic disorder of long-chain fatty acid oxidation caused by a homozygous or compound heterozygous mutation in the SLC25A20 gene. The SLC25A20 gene encodes carnitine-acylcarnitine translocase (CACT), one of the components of the carnitine cycle that mediates the transport of acylcarnitines of different length across the mitochondrial inner membrane from the cytosol to the mitochondrial matrix for their oxidation by the mitochondrial fatty acid-oxidation pathway.

[0222] Individuals with CACTD exhibit hypoketotic hypoglycemia under fasting conditions, hyperammonemia, elevated serum creatine kinase and transaminases, dicarboxylic aciduria, very low free carnitine, and abnormal acylcarnitine profile with marked elevation of the long-chain acylcarnitines. Additional features include neurologic abnormalities, cardiomyopathy and arrhythmias, skeletal muscle damage, and liver dysfunction. Most patients become symptomatic in the neonatal period with a rapidly progressive deterioration and a high mortality rate. However, presentations at a later age with a milder phenotype have been reported.

Cartilage-hair Hypoplasia

[0223] Cartilage-hair hypoplasia, a form of short-limbed dwarfism due to skeletal dysplasia, is caused by mutations in the RMRP gene. RMRP encodes an RNA with endoribonuclease activity that cleaves mitochondrial RNA complementary to light chain of displacement loop. Clinical symptoms include short stature, joint hyperextensibility, metaphyseal dysplasia, hypoplastic sparse hair, neuronal dysplasia, megacolon, malabsorption, increased risk of lymphoma & skin neoplasm, susceptibility to chickenpox, lymphopenia, neutropenia, and hypoplastic macrocytic anemia.

Cerebrotendinous Xanthomatosis

[0224] Cerebrotendinous xanthomatosis (CTX), also known as Van Bogaer-Scherer-Epstein disease, is an autosomal recessive lipid-storage disorder caused by the deficient activity of mitochondrial sterol 27-hydroxylase (CYP27A1). CTX is associated with mutations in the CYP27A1 gene, which encodes sterol 27-hydroxylase. CTX is characterized by the formation of xanthomatous lesions in many tissues, particularly in the brain, eye lens, and tendons resulting in progressive neurologic dysfunction, premature atherosclerosis, and

cataracts. Cholestanol, the 5-alpha-dihydro derivative of cholesterol, is enriched relative to cholesterol in all tissues. A diagnosis of CTX is typically made by demonstrating that cholestanol is present in abnormal amounts in the serum and tendon in suspected affected individuals.

Congenital Adrenal Hyperplasia

[0225] Congenital adrenal hyperplasia (CAH) comprises a group of monogenic autosomal recessive disorders caused by an enzyme deficiency in steroid biosynthesis. All of the adrenal hyperplasia syndromes are examples of mixed hypo- and hyperadrenocorticism. CAH is associated with 11-beta-hydroxylase deficiency caused by a mutation in the CYP11B1 gene. The CYP11B1 gene encodes 11-beta-hydroxylase, which functions primarily in the mitochondria in the zona fasciculata of the adrenal cortex to convert 11-deoxycortisol to cortisol and 11-deoxycorticosterone to corticosterone. CAH due to 11-beta-hydroxylase deficiency results in androgen excess, virilization, and hypertension. The defect causes decreased cortisol and corticosterone synthesis in the zona fasciculata of the adrenal gland, resulting in the accumulation of 11-deoxycortisol and 11-deoxycorticosterone.

Congenital Muscular Dystrophy with Mitochondrial Structural Abnormalities (Megaconial) (MDCMC)

[0226] Megaconial type congenital muscular dystrophy is caused by homozygous or compound heterozygous mutations in the choline kinase beta (CHKB) gene. This form of autosomal recessive congenital muscular dystrophy is characterized by early-onset muscle wasting and mental retardation. Some patients develop fatal cardiomyopathy. Muscle biopsy shows peculiar enlarged mitochondria that are prevalent toward the periphery of the fibers but are sparse in the center. Additional clinical symptoms include hypotonia, progressive weakness, delayed walking, small head circumference, elevated creatine kinase, muscle necrosis, and increased endomysial connective tissue.

Cerebral Creatine Deficiency Syndrome-3

[0227] Cerebral creatine deficiency syndromes (CCDS) comprise a group of inborn errors of creatine metabolism and include the X-linked creatine transporter (SLC6A8) deficiency (CCDS1) and the two autosomal recessive creatine biosynthesis disorders, guanidinoacetate methyltransferase (GAMT) deficiency (CCDS2) and L-arginine:glycine amidinotransferase (AGAT or GATM) deficiency (CCDS3). Cerebral creatine deficiency syndrome-3 (CCDS3)

is associated with the following AGAT mutations: Ala97ValfsX11; Trp149X; Arg169X; Tyr203Ser; and Met371AsnfsX6. AGAT is localized to the mitochondrial intermembrane space.

[0228] These disorders are characterized by developmental delay/regression, mental retardation, severe depressive and cognitive speech disturbances, seizures, and depletion of creatine/phosphocreatine levels in the brain. Additional manifestations include muscular hypotonia and movement disorder (mainly extrapyramidal). The characteristic biochemical hallmark of all CCDS is cerebral creatine deficiency as detected by proton magnetic resonance spectroscopy (H-MRS). Increased levels of guanidinoacetate in body fluids are indicative of GAMT deficiency, whereas reduced guanidinoacetate levels are indicative of AGAT deficiency. An elevated urinary creatine/creatinine ratio is associated with SLC6A8 deficiency.

Deafness

1. Maternal Nonsyndromic Deafness

[0229] Mutations in mitochondrial DNA (mtDNA) have been found to be associated with nonsyndromic sensorineural hearing loss. Mitochondrially inherited nonsyndromic sensorineural deafness can be caused by mutations in any 1 of several mitochondrial genes, including MTRNR1, MTTS1, MTCO1, MTTH, MTND1, and MTTI. Matrilineal relatives within and among families carrying certain pathogenic mitochondrial mutations exhibit a wide range of penetrance, severity, and age of onset of hearing loss, indicating that the mitochondrial mutations by themselves are not sufficient to produce a deafness phenotype. Modifier factors, such as nuclear and mitochondrial genes, or environmental factors, such as exposure to aminoglycosides, appear to modulate the phenotypic manifestations.

2. Maternal Syndromic Deafness

[0230] Mitochondrially inherited syndromic sensorineural deafness can be caused by mutations in any 1 of several mitochondrial genes (including MTTL1, MTTS1, MTTS2, MTTL, MTTK, MTTQ), large (> 1 kb) heteroplasmic deletions, or large (> 1 kb) heteroplasmic partial duplications.

3. Sporadic Syndromic Deafness

[0231] Sporadic syndromic deafness can be caused by single large mtDNA deletion or mutations in MTCO1. Clinical symptoms associated with MTCO1-induced syndromic deafness include cataracts, progressive sensorineural deafness, myopathy, ataxia, myoclonic epilepsy, visual loss, optic atrophy, reduced COX activity, cerebellar atrophy, and bilateral small symmetrical nodular hyperintensities. Mutations in MTCO1 can result in sideroblastic anemia, exercise intolerance and LHON.

4. Autosomal Dominant Deafness-64 (DFNA64)

[0232] Autosomal dominant deafness-64 (DFNA64) is caused by heterozygous mutations in the DIABLO gene. The age at onset ranges between 12 and 30 years (average age of 22). The severity of hearing impairment ranges from severe to moderate to mild and correlates with age. High frequency tinnitus was reported in 73% of affected individuals at the onset of hearing loss.

5. Deafness-Dystonia-Dementia Syndromes

[0233] Mohr-Tranebjaerg Syndrome and Jensen Syndrome have been found to be caused by mutations in the TIMM8A (DDP) gene, which aid in the importation of metabolite transporters from cytoplasm to mitochondrial inner membrane. Clinical symptoms of Mohr-Tranebjaerg Syndrome (Deafness-dystonia Syndrome) include progressive sensory-neural hearing loss, myopia, reduced visual acuity, constricted visual fields, retinal change, dystonia, mental deficiency, and cortical blindness. Clinical symptoms of Jensen Syndrome include blindness, optic atrophy, sensorineural hearing loss, dementia, CNS calcifications, and muscle wasting.

[0234] Mutations in the chaperonin HSP60 also impact mitochondrial protein importation and may lead to hereditary spastic paraplegia.

6. Dystonia, Deafness with Leigh-like Syndrome (MEGDEL)

[0235] 3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like Syndrome (MEGDEL) is an autosomal recessive disorder characterized by childhood onset of delayed psychomotor development or psychomotor regression, sensorineural deafness, spasticity or dystonia, and increased excretion of 3-methylglutaconic acid. MEGDEL is caused by homozygous or compound heterozygous mutations in the SERAC1 gene, which

plays a role in phospholipid exchange and intracellular cholesterol trafficking. Brain imaging of affected subjects shows cerebral and cerebellar atrophy as well as lesions in the basal ganglia reminiscent of Leigh Syndrome. Clinical symptoms include hypotonia, encephalopathy (Leigh-like Syndrome), mental retardation, sensorineural deafness, spasticity, dystonia, hepatopathy, increased serum lactate and alanine, hyperammonemia, 3-Methylglutaconic aciduria, high transaminases, coagulopathy, high serum α-fetoprotein, mitochondrial oxidative phosphorylation defects, abnormal mitochondria, abnormal phosphatidylglycerol and cardiolipin profiles in fibroblasts, and abnormal accumulation of unesterified cholesterol within cells.

7. Reticular Dysgenesis

[0236] Reticular dysgenesis is one of the rarest and most severe forms of combined immunodeficiency and is caused by a homozygous or compound heterozygous mutation in the mitochondrial adenylate kinase-2 gene (AK2). Reticular dysgenesis is characterized by bilateral sensorineural deafness, congenital agranulocytosis, lymphopenia, and lymphoid and thymic hypoplasia with absent cellular and humoral immunity functions.

8. Steroid-resistant Nephrotic Syndrome & Sensorineural Hearing Loss (COQ10D6)

[0237] Primary coenzyme Q10 deficiency-6 (COQ10D6) is an autosomal recessive disorder characterized by onset in infancy of severe progressive nephrotic syndrome resulting in end-stage renal failure and sensorineural deafness. COQ10D6 is caused by homozygous or compound heterozygous mutations in the COQ6 gene. Renal biopsy usually shows focal segmental glomerulosclerosis (FSGS). Clinical features include bilateral sensorineural deafness and steroid-resistant nephrotic syndrome.

9. Cataracts, Growth hormone Deficiency, Sensory Neuropathy, Sensorineural Hearing Loss, Skeletal Dysplasia (CAGSSS)

[0238] Mutations in isoleucyl-tRNA synthetase 2 (IARS2), an aminoacyl-tRNA synthetase, results in CAGSSS. Clinical symptoms include sensorineural deafness, cataracts, skeletal dysplasia, facial dysmorphism, short stature, hip disorders, scoliosis, cervical stenosis, C2-odontoid hypoplasia, spondylo-epiphyseal dysplasia, sensory loss polyneuropathy, growth hormone deficiency, and pituitary adenohypophysis atrophy. Alternate IARS2 syndromes include Leigh Syndrome.

Diabetes

[0239] Diabetes may arise as a result of mutations in MTTL, MTTK, MTTE, MTTS2, mitochondrial elongation factor G2 (GFM2), single large mtDNA deletions, and large scale mtDNA tandem duplications. Diabetes is usually observed in patients affected with Kearns-Sayre Syndrome, Wolfram Syndrome, Friedreich's ataxia and Metabolic Syndrome in obesity.

Dimethylglycine Dehydrogenase Deficiency

[0240] Dimethylglycine dehydrogenase deficiency (DMGDHD) is an autosomal recessive glycine metabolism disorder characterized by chronic muscle fatigue, elevated serum levels of creatine kinase, and a fishlike body odor. DMGDHD is also characterized by an increase of N,N-dimethylglycine (DMG) in serum and urine. DMGDHD is associated with mutations in the DMGDH gene, which encodes dimethylglycine dehydrogenase (DMGDH), a mitochondrial matrix flavoprotein that catalyzes the oxidative demethylation of dimethylglycine to form sarcosine. DMGDH has been identified as a monomer in the mitochondrial matrix where it uses flavin adenine dinucleotide and folate as cofactors.

Encephalopathies

1. Multiple Mitochondrial Encephalopathy

[0241] Multiple mitochondrial encephalopathies can result from Multiple Mitochondrial Dysfunctions Syndrome-1 (MMDS1), Multiple Mitochondrial Dysfunctions Syndrome-2 (MMDS2), and Multiple Mitochondrial Dysfunctions Syndrome-3 (MMDS3). Multiple mitochondrial dysfunctions syndrome is a severe autosomal recessive disorder of systemic energy metabolism, resulting in weakness, respiratory failure, lack of neurologic development, lactic acidosis, and early death. MMDS1 be caused by a homozygous or compound heterozygous mutation in the NFU1 gene. MMDS2 can be caused by homozygous mutation in the BOLA3 gene. MMDS3 can be caused by homozygous mutation in the IBA57 gene.

2. Encephalopathies Associated with Mitochondrial Complex I Deficiency

[0242] Encephalopathy can arise as a result of mitochondrial Complex I deficiencies. Complex I deficiencies leading to encephalopathy are associated with mutations in the following genes: NDUFA1, NDUFA11, C6ORF66, VARS2, NDUFA12L, NDUFS1, NDUFV1, NUBPL, and NDUFV2.

3. Childhood Leukoencephalopathy and Complex II Deficiency

[0243] Childhood leukoencephalopathy associated with mitochondrial Complex II deficiency can be caused by mutations in the SDHAF1 gene, which encodes succinate dehydrogenase complex assembly factor 1.

4. Encephalopathies Associated with Mitochondrial Complex III Deficiency

[0244] Encephalopathy can arise as a result of mitochondrial Complex III deficiencies. Complex III deficiencies leading to encephalopathy are associated with mutations in the following genes: UQCRQ, UQCC2, LYRM7, and UQCRC2.

5. Encephalopathies Associated with Mitochondrial Complex IV Deficiency

[0245] Encephalopathies associated with mitochondrial Complex IV deficiency include encephalocardiomyopathies due to mutations in the MTO1 gene and/or C12ORF62 genes, encephalomyopathies due to mutations in the FASTKD2 and/or AIFM1 genes, and neonatal hepatoencephalopathy due to mutations in the SCO1 gene.

6. Encephalopathies Associated with Mitochondrial Complex V Deficiency

[0246] Encephalopathies associated with mitochondrial Complex V deficiency include neonatal encephalopathy, which is caused by mutation in the ATP5A1 gene, and neonatal encephalocardiomyopathy, which is caused by mutation in the TMEM70 gene.

7. Hyperammonemia due to Carbonic Anhydrase VA Deficiency

[0247] Hyperammonemia due to carbonic anhydrase VA deficiency (CA5AD) is caused by homozygous mutation in the CA5A gene. The disorder is characterized clinically by acute onset of encephalopathy in infancy or early childhood. Biochemical evaluation shows multiple metabolic abnormalities, including metabolic acidosis and respiratory alkalosis. Other abnormalities include hypoglycemia, increased serum lactate and alanine, and evidence of impaired provision of bicarbonate to essential mitochondrial enzymes. Apart from episodic acute events in early childhood, the disorder showed a relatively benign course.

8. Early Infantile Epileptic Encephalopathy-3

[0248] Early infantile epileptic encephalopathy-3 (EIEE3) is caused by homozygous mutation in the SLC25A22 gene. EIEE3 is characterized by onset during the first months of life of erratic refractory seizures, usually myoclonic. The prognosis is poor, and most

children with the condition either die within 1 to 2 years after birth or survive in a persistent vegetative state. The EEG pattern often shows a suppression-burst pattern with high-voltage bursts of slow waves mixed with multifocal spikes alternating with isoelectric suppression phases.

9. 2,4-Dienoyl-CoA Reductase Deficiency

[0249] 2,4-Dienoyl-CoA reductase deficiency (DECRD) is caused by a homozygous mutation in the NADK2 gene. DECR deficiency is a rare autosomal recessive inborn error of metabolism resulting in mitochondrial dysfunction. Affected individuals have a severe encephalopathy with neurologic and metabolic dysfunction beginning in early infancy. Laboratory studies show decreased activity of the mitochondrial NADP(H)-dependent enzymes DECR1 and AASS, resulting in increased C10:2-carnitine levels and hyperlysinemia.

10. Infection-induced Acute Encephalopathy-3

[0250] Infection-induced acute encephalopathy-3 (IIAE3), also known as acute necrotizing encephalopathy, is caused by heterozygous mutation in the RANBP2 gene. Affected individuals typically present with IIAE3 following febrile illness.

11. Ethylmalonic Encephalopathy

[0251] Ethylmalonic encephalopathy (EE) is caused by a homozygous or compound heterozygous mutation in the ETHE1 gene, which encodes a mitochondrial matrix protein. Ethylmalonic encephalopathy is an autosomal recessive severe metabolic disorder of infancy affecting the brain, gastrointestinal tract, and peripheral vessels. The disorder is characterized by neurodevelopmental delay and regression, prominent pyramidal and extrapyramidal signs, recurrent petechiae, orthostatic acrocyanosis, and chronic diarrhea. Brain MRI shows necrotic lesions in deep gray matter structures. Death usually occurs in the first decade of life.

12. Hypomyelinating Leukodystrophy

[0252] Hypomyelinating leukodystrophy (HLD4), also known as mitochondrial Hsp60 chaperonopathy, is caused by mutation in the HSPD1 gene. Affected individuals experience a form of severe hypomyelinating leukoencephalopathy. Age of onset typically occurs between birth and three month. The disorder is characterized by hypotonia, nystagmus, and

psychomotor developmental delay, followed by appearance of prominent spasticity, developmental arrest, and regression.

Exocrine Pancreatic Insufficiency, Dyserythropoietic Anemia and Calvarial Hyperostosis

[0253] Exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis is caused by mutations in cytochrome c oxidase, Subunit IV, Isoform 2 (COX4I2). Clinical features include exocrine pancreatic insufficiency, steatorrhea, malabsorption of lipid-soluble vitamins, calvarial hyperostosis, delayed bone age, osteopenia and dyserythropoietic, megaloblastic anemia.

Glutaric Aciduria Type 1

[0254] Glutaric aciduria type 1 (GA-1), also known as glutaric acidemia, is an autosomal recessive disorder characterized by episodes of severe brain dysfunction, spasticity, hypotonia, dystonia, seizures, and developmental delays. GA-1 is associated with mutations in the GCDH gene causing a deficiency of glutaryl-CoA dehydrogenase (GCDH) and leading to an accumulation of glutaric and 3-hydroxyglutaric acids and secondary carnitine deficiency. Elevated urine C5DC serves as a marker for the detection of GA-1.

[0255] GCDH is an acyl dehydrogenase that catalyzes the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA and CO_2 in the degradative pathway of L-lysine, L-hydroxylysine, and L-tryptophan metabolism. The enzyme exists as a homotetramer of 45-kD subunits in the mitochondrial matrix. Deficiencies in GCDH lead to an accumulation of L-lysine, L-hydroxylysine, L-tryptophan, and their metabolites.

Glycine Encephalopathy

[0256] Glycine encephalopathy (GCE), also known as nonketotic hyperglycinemia (NKH), is an inborn error of glycine metabolism caused by a deficiency of the glycine cleavage system. GCE is characterized by abnormally high levels of glycine leading to a progressive lethargy, feeding difficulties, hypotonia, dystonia, and respiratory distress. The enzyme system for cleavage of glycine, which is confined to the mitochondria, is composed of four protein components: P protein (a pyridoxal phosphate-dependent glycine decarboxylase), H protein (a lipoic acid-containing protein), T protein (a tetrahydrofolate-requiring enzyme), and L protein (a lipoamide dehydrogenase). GCE may be caused by a defect in the H, P, or T proteins.

Hepatic Failure

[0257] Acute infantile liver failure is caused by mutations in tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (TRMU), which encodes a mitochondria-specific tRNA-modifying enzyme. Age of onset for this disorder is usually between 1 to 4 months and is characterized by hepatic failure, irritability, poor feeding, and vomiting. Clinical features include jaundiced sclerae, distended abdomen, hepatomegaly, lethargy, coagulopathy, low albumin, direct hyperbilirubinemia, metabolic acidosis, hyperlactatemia, high α-fetoprotein, high phenylalanine, tyrosine, methionine, glutamine and alanine in plasma, high lactate, phenylalanine and tyrosine metabolites, ketotic dicarboxylic and 3-hydroxydicarboxylic aciduria, and reduced Complex I, III and IV activity.

[0258] Hepatic failure with hyperlactatemia is caused by mutations in DGUOK, POLG, and MPV17.

2-Hydroxyglutaric Aciduria

[0259] 2-Hydroxyglutaric aciduria is an autosomal recessive neurometabolic disorder characterized by developmental delay, epilepsy, hypotonia, and dysmorphic features. Mutations in the D2HGDH gene, encoding D-2-hydroxyglutarate dehydrogenase (D2HGDH) are associated with D-2-hydroxyglutaric aciduria (D-2-HGA) type I. D2HGDH is a mitochondrial enzyme belonging to the FAD-binding oxidoreductase/transferase type 4 family that is active in liver, kidney, heart, and brain where it converts D-2-hydroxyglutarate (D-2-HG) to 2-ketoglutarate. Mutations in the IDH2 gene, encoding isocitrate dehydrogenase 2 (IDH2) are associated with D-2-HGA type II. IDH2 is a mitochondrial NADP-dependent isocitrate dehydrogenase that catalyzes oxidative decarboxylation of isocitrate to alpha-ketoglutarate, producing NADPH.

[0260] Another form of 2-hydroxyglutaric aciduria, L-2-hydroxyglutaric aciduria (L-2-HGA) is associated with mutations in the L2HGDH gene, encoding L-2-hydroxyglutarate dehydrogenase, an FAD-dependent mitochondrial enzyme that oxidizes L-2-hydroxyglutarate to alpha-ketoglutarate. L-2-HGA particularly affects the cerebellum, resulting in balance and muscle coordination abnormalities. Clinical manifestations of infantile onset L-2-HGA include ataxia, mental retardation, macrocephaly, a potential increased risk of brain neoplasm, leukodystrophy, and the presence of L-2-hydroxyglutaric acid in the urine and

cerebrospinal fluid. Adult-onset L-2-HGA is associated with a c.959delA mutation and causes movement disorder, tremor, and saccades.

[0261] Combined D,L-2-hydroxyglutaric aciduria (D,L-2HGA) is characterized by neonatal-onset encephalopathy with severe muscular weakness, intractable seizures, respiratory distress, and lack of psychomotor development leading to early death.

3-Hydroxyacyl-CoA Dehydrogenase Deficiency

[0262] 3-Hydroxyacyl-CoA dehydrogenase deficiency, also known as HADH deficiency, is an autosomal recessive metabolic disorder, resulting from mutations in the HADH gene. The 3-Hydroxyacyl-CoA dehydrogenase protein functions in the mitochondrial matrix to catalyze the oxidation of straight-chain 3-hydroxyacyl-CoAs as part of the beta-oxidation pathway. Human HADH encodes a deduced 314-amino acid protein comprising a 12-residue mitochondrial import signal peptide and a 302-residue HADH protein with a calculated molecular mass of 34.3 kD. 3-Hydroxyacyl-CoA dehydrogenase has a preference for medium chain substrates, whereas short chain 3-hydroxyacyl-CoA dehydrogenase (SCADH) acts on a variety of substrates, including steroids, cholic acids, and fatty acids with a preference for short chain methyl-branched acyl-CoAs. Mutations in HADH cause one form of familial hyperinsulinemic hypoglycemia (FHH). FHH is the most common cause of persistent hypoglycemia in infancy.

Hypercalcemia Infantile

[0263] Hypercalcemia infantile is an autosomal recessive disorder characterized by severe hypercalcemia, failure to thrive, vomiting, dehydration, and nephrocalcinosis. Hypercalcemia infantile is associated with homozygous or compound heterozygous mutations in the CYP24A1 gene. 24-Hydroxylase (CYP24A1) is a mitochondrial enzyme found mainly in the kidney, bone and intestine, and is likely present in all cells that express the vitamin D receptor. CYP24A1 is a 514-amino acid protein with a complex structure of α helices and β strands. It interacts with the mitochondrial membrane, adrenodoxin, heme, and vitamin D molecules. Disruption of this structure impairs the function of the enzyme. Tight control of the vitamin D system requires inactivation of its active compound 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D3$) through 24-hydroxylation by means of the CYP24A1 enzyme and degradation to calcitroic acid.

Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) Syndrome

[0264] HHH Syndrome is an autosomal recessive early onset (infancy to 18 years) disorder caused by mutations in the SLC25A15 gene, which encodes the mitochondrial ornithine transporter. Patients with HHH exhibit partial impairment of uptake of ornithine by mitochondria. Symptoms include mental retardation and myoclonic seizures associated with hyperornithinemia, hyperammonemia, and homocitrullinemia, progressive spastic paraparesis, protein intolerance, stuporous episodes, cerebellar ataxia, muscular weakness in both legs, myoclonus, lethargy, dysmetria, dysdiadochokinesis, scanning speech, learning difficulties, buccofaciolingual dyspraxia, episodic vomiting, retinal depigmentation, and chorioretinal thinning.

Immunodeficiency with Hyper-IgM Type 5

[0265] Immunodeficiency with hyper-IgM type 5 (HIGM5) is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the gene encoding uracil-DNA glycosylase (UNG). HIGM5 is characterized by defective normal or elevated serum IgM concentrations in the presence of diminished or absent IgG, IgA, and IgE concentrations, indicating a defect in the class-switch recombination (CSR) process. UNG removes uracil in DNA resulting from deamination of cytosine or replicative incorporation of dUMP instead of dTMP, thereby suppressing GC-to-AT transition mutations. The UNG gene encodes two isoforms that are individually targeted to the mitochondria and nucleus. The mitochondrial isoform is referred to as UNG1, UDG1, or UDG1M, and the nuclear isoform is referred to as UNG2, UDG1A, or UDG1N. HIGM5 is associated with mutations in the UNG gene. Patients with HIGM5 typically experience recurrent bacterial infections and often exhibit lymphoid hyperplasia.

Inflammatory Myopathies

1. Inclusion Body Myositis (IBM)

[0266] IBM or Inflammatory Myopathy with Vacuoles, Aggregates and Mitochondrial Pathology (IM-VAMP) is a sporadic progressive condition that typically manifests in 9 out of 10^6 individuals at > 50 years of age. Clinical features include proximal and distal weakness, dysphagia, Inflammatory myopathy with Mitochondrial pathology (PM-Mito), respiratory failure, aspiration, cachexia, muscle atrophy, diminished tendon reflexes, polyneuropathy, inflammation, muscle fiber hypertrophy, rimmed vacuoles with granular material & filaments

(β-Amyloid, Desmin; Ubiquitin; Transglutaminases 1 & 2), aggregates (stain for SMI-31 antibody, LC-3, β-amyloid, VCP, ubiquitin, αB-crystallin), COX deficient and SDH + muscle fibers, multiple mtDNA deletions, cricopharyngeus dysfunction, fatty infiltration, elevated transglutaminase activity, MHC I upregulation in muscle fibers, and increased frequency of non-organ specific autoantibodies.

[0267] Variant syndromes include autosomal dominant IBM and polymyositis with mitochondrial pathology.

2. Inflammatory Myopathy + Mitochondrial Pathology in Muscle (IM-Mito)

[0268] The age of onset for IM-Mito ranges from 43 to 71 years. Disease progression is slower than IBM. Symptoms include proximal and distal weakness, elevated serum creatine kinase, COX-negative and SDH-positive muscle fibers, endomysial inflammation, focal invasion of muscle fibers by inflammatory cells, multiple mtDNA deletions, and LC-3 and/or αB-crystallin aggregates in muscle fibers.

3. Granulomatous Myopathies with Anti-mitochondrial Antibodies

[0269] Granulomatous myopathies with anti-mitochondrial antibodies account for 11% of inflammatory myopathies in Tokyo and typically manifests between 33 to 72 years of age. Clinical features include primary biliary cirrhosis, cardiac arrhythmias, muscle weakness, atrophy, respiratory defects, skin rash, elevated anti-mitochondrial autoantibodies, high serum creatine kinase, elevated alkaline phosphatase, endomysial fibrosis, necrosis and regeneration, inflammation, granulomas and MHC I upregulation in muscle fibers.

[0270] Other inflammatory myopathies include, but are not limited to, necrotizing myopathy with pipestem capillaries, myopathy with deficient chondroitin sulfate C in skeletal muscle connective tissue, benign acute childhood myositis, idiopathic orbital myositis, masticator myopathy, hemophagocytic lymphohistiocytosis, infection-associated myositis, Facioscapulohumeral dystrophy (FSH), Limb-Girdle dystrophy, familial idiopathic inflammatory myopathy, Schmidt Syndrome (Diabetes mellitus, Addison disease, Myxedema), TNF receptor-associated Periodic Syndrome (TRAPS), focal myositis, autoimmune fasciitis, Spanish toxic oil-associated fasciitis, Eosinophilic fasciitis, Macrophagic myofasciitis, Graft-vs-host disease fasciitis, Eosinophilia-myalgia Syndrome, and perimyositis.

Isovaleric Acidemia

[0271] Isovaleric acidemia (IVA), also known as isovaleric aciduria, is an autosomal recessive inborn error of leucine metabolism caused by a deficiency of the mitochondrial enzyme isovaleryl-CoA dehydrogenase (IVD) resulting in the accumulation of derivatives of isovaleryl-CoA such as isovaleric acid, which is toxic to the central nervous system. There are two forms of IVA. The acute neonatal form leads to pernicious vomiting, massive metabolic acidosis, and rapid death. The chronic form results in periodic attacks of severe ketoacidosis with asymptomatic intervening periods. Symptoms of IVA include convulsions, lethargy, dehydration, moderate hepatomegaly, depressed platelets and leukocytes, and a distinctive odor resembling that of sweaty feet.

Kearns-Sayre Syndrome

[0272] Kearnes-Sayre Syndrome (KSS), also known as oculocranisomatic disorder or oculocraniosomatic neuromuscular disorder with ragged red fibers, is a mitochondrial myopathy that is caused by various mitochondrial deletions. Single large mtDNA deletions (2 to 8 kb) account for 80% of KSS mutations. The mtDNA deletions that cause KSS result in the impairment of oxidative phosphorylation and a decrease in cellular energy production. In most instances, KSS arises from sporadic somatic mutations occurring after conception. Rarely, the mutation is transmitted through maternal inheritance.

[0273] Clinical features include progressive external ophthalmoplegia, pigmentary degeneration of retina (retinitis pigmentosa), heart block, mitochondrial myopathy, limitation or absence of movement in all fields of gaze, ptosis, dysphagia, weight loss, weakness, occasional fatigue or pain on exertion, sensory-motor polyneuropathy, stroke, reduced respiratory drive, hearing loss, ataxia, dementia, or impaired intellect, spasticity, growth hormone deficiency, increased tendon reflexes, endocrinopathies, glucose intolerance, hypothyroidism, hypoparathyroidism, short stature, ragged red fibers, variation in muscle fiber size, lactic acidosis, high CSF protein, low 5-methyltetrahydrofolate (5-MTHF) in CSF, high homovanillic acid (HVA) in CSF, abnormal choroid plexus function, basal ganglia calcifications, cerebral and cerebellar atrophy, and status spongiosis in gray and white matter.

[0274] Another variant syndrome related to KSS, or other disorders having a single large mtDNA deletion include 2-oxoadipic aciduria and 2-aminoadipic aciduria. Affected patients exhibit episodes of ketosis and acidosis, and may experience coma.

Limb-girdle Muscular Dystrophy (LGMD) Syndromes

[0275] LGMD1 is an autosomal dominant disorder characterized by adult onset of proximal muscle weakness, beginning in the hip girdle region and later progressing to the shoulder girdle region. Distal muscle weakness may occur later. Autosomal dominant limb-girdle muscular dystrophy (LGMD) type 1A is caused by a heterozygous mutation in the gene encoding myotilin (TTID). Other forms of autosomal dominant LGMD include LGMD1B, caused by mutations in the LMNA gene; LGMD1C, caused by mutations in the CAV3 gene; LGMD1E, caused by mutations in the DNAJB6 gene; LGMD1F, caused by mutations in the TNPO3 gene; LGMD1G, which maps to chromosome 4q21; and LGMD1H, which maps to chromosome 3p25-p23. The symbol LGMD1D was formerly used for a disorder later found to be the same as desmin-related myopathy.

[0276] Autosomal recessive forms of LGMD include LGMD2A, caused by mutations in Calpain-3; LGMD2B, caused by mutations in Dysferlin; LGMD2C, caused by mutations in γ-Sarcoglycan; LGMD2D, caused by mutations in α-Sarcoglycan; LGMD2E, caused by mutations in β-Sarcoglycan; LGMD2F, caused by mutations in δ-Sarcoglycan; LGMD2G, caused by mutations in Telethonin; LGMD2H, caused by mutations in TRIM32; LGMD2I (MDDGC5), caused by mutations in FKRP; LGMD2J, caused by mutations in Titin; LGMD2K (MDDGC1), caused by mutations in POMT1; LGMD2L, caused by mutations in ANO5; LGMD2M (MDDGC4), caused by mutations in Fukutin; LGMD2N (MDDGC2), caused by mutations in POMT2; LGMD2O (MDDGC3), caused by mutations in POMGnT1; LGMD2P (MDDGC9), caused by mutations in DAG1; LGMD2Q, caused by mutations in Plectin 1f; LGMD2R, caused by mutations in Desmin; and LGMD2S, caused by mutations in TRAPPC11.

Leukodystrophy

[0277] Mutations in COX6B1, which encodes a Complex IV structural subunit, can result in mitochondrial complex IV deficiency. Symptoms include muscle weakness, pain, unsteady gait, visual loss, progressive neurological deterioration, cognitive decline, leukodystrophic brain changes, seizures, ataxia, increased serum and CSF lactate and decreased COX activity in muscle.

[0278] Mutations in Apoptogenic protein 1 (APOPT1), which mediates mitochondria-induced cell death in vascular smooth muscle cells, can result in cavitating leukodystrophy.

This disorder typically manifests between the ages of 2 to 5 years. Additional clinical features include spastic tetraparesis, ataxia, sensory-motor polyneuropathy, reduced cognition, reduced COX staining, large mitochondria with osmophilic inclusions, and reduced Complex IV activity.

[0279] Mutations in SDHB can result in leukodystrophy, which usually presents at 1 year of age. Clinical features include loss of motor skills, leukodystrophy in deep white matter and corpus callosum, and reduced Complex II activity.

[0280] Leukodystrophy may arise as a result of large mtDNA deletions. Clinical features include progressive ataxia, bulbar palsy, white-matter lesions in occipital to parietal lobes, and high CSF lactate.

Maple Syrup Urine Disease

[0281] Maple syrup urine disease (MSUD) can be caused by homozygous or compound heterozygous mutations in at least 3 genes: BCKDHA, BCKDHB, and DBT. These genes encode 2 of the catalytic components of the branched-chain alpha-keto acid dehydrogenase complex (BCKDC), which catalyzes the catabolism of the branched-chain amino acids, leucine, isoleucine, and valine. Maple syrup urine disease caused by a mutation in the E1-alpha subunit gene is referred to as MSUD type IA; that caused by a mutation in the E1-beta subunit gene as type IB; and that caused by defect in the E2 subunit gene as type II. Mutations in the third component, E3 (DLD), on chromosome 7q31, cause an overlapping but more severe phenotype known as dihydrolipoamide dehydrogenase deficiency (DLDD). DLD deficiency is sometimes referred to as MSUD3.

[0282] Clinical features of maple syrup urine disease include mental and physical retardation, neuropathy, ataxia, dystonia, athetosis, dysarthria, weakness, ophthalmoplegia, hearing loss, drowsiness, seizures, feeding problems, reduced tendon reflexes, sensory loss/pain, endoneurial edema, high lactic acid, and a maple syrup odor to the urine. The keto acids of the branched-chain amino acids are present in the urine, resulting from a block in oxidative decarboxylation. There are 5 clinical subtypes of MSUD: the 'classic' neonatal severe form, an 'intermediate' form, an 'intermittent' form, a 'thiamine-responsive' form, and an 'E3-deficient with lactic acidosis' form. All of these subtypes can be caused by mutations in any of the 4 genes mentioned above, except for the E3-deficient form, which is caused only by a mutation in the E3 gene.

3-Methylcrotonyl-CoA Carboxylase Deficiency

[0283] 3-Methylcrotonyl-CoA carboxylase (MCC), deficiency also known as 3-methylcrotonylglycinuria, is an autosomal recessive disorder of leucine catabolism with a variable phenotype, ranging from neonatal onset with severe neurological involvement to asymptomatic adults. Common symptoms include feeding difficulties, recurrent episodes of vomiting and diarrhea, lethargy, and hypotonia. MCC is a heteromeric biotin-dependent mitochondrial enzyme composed of alpha subunits and smaller beta subunits, encoded by MCC1 and MCC2, respectively. MCC is essential for the catabolism of leucine.

Methylmalonic Aciduria

[0284] Methylmalonic aciduria (MMA), also known as methylmalonyl-CoA epimerase deficiency, is an autosomal recessive disorder characterized by progressive encephalopathy, dehydration, developmental delays, failure to thrive, lethargy, seizures, and vomiting. MMA is caused by mutations in the MUT, MMAA, MMAB, MMADHC, and MCEE genes. The long-term effects of MMA depend on which gene is mutated and the severity of the mutation.

[0285] Mutations in the MUT gene cause a deficiency of methylmalonyl-CoA mutase (MUT), which is a vitamin B12-dependent mitochondrial enzyme that catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA. Mutations in the MUT gene lead to a toxic accumulation of methylmalonic acid in the blood. The proteins encoded by the MMAA, MMAB, and MMADHC genes are required for the proper function of MUT. Mutations affecting these genes can impair the activity of MUT, leading to methylmalonic aciduria. Mutations in the MCEE gene, which encodes methylmalonyl CoA epimerase, lead to a mild form of methylmalonic aciduria.

Miller Syndrome

[0286] Miller Syndrome, also known as postaxial acrofacial dystosis, is an autosomal recessive disorder characterized by severe micrognathia, cleft lip and/or palate, hypoplasia or aplasia of the postaxial elements of the limbs, coloboma of the eyelids, and supernumerary nipples. Miller Syndrome is associated with mutations in the DHODH gene encoding dihydroorotate (DHO) dehydrogenase. Dihydroorotate dehydrogenase catalyzes the fourth enzymatic step in de novo pyrimidine biosynthesis. DHO dehydrogenase is a monofunctional protein located on the outer surface of the inner mitochondrial membrane.

mtDNA Depletion Syndrome-2 (MTDPS2)

[0287] Mitochondrial DNA Depletion Syndrome-2 (MTDPS2) is an autosomal recessive disorder characterized primarily by childhood onset of muscle weakness associated with depletion of mtDNA in skeletal muscle. MTDPS2 is caused by homozygous or compound heterozygous mutations in the nuclear-encoded mitochondrial thymidine kinase gene (TK2). There is wide clinical variability; some patients have onset in infancy and show a rapidly progressive course with early death due to respiratory failure, whereas others have later onset of a slowly progressive myopathy.

[0288] Clinical features include gait impairment, hypotonia, weakness, respiratory failure, paralysis, gynecomastia, myopathy, chronic partial denervation, mtDNA depletion, reduced Complex I, III, IV and V activity, and elevated plasma lactate.

[0289] Variant TK2 syndromes include spinal muscular atrophy syndrome, rigid spine syndrome, and severe myopathy with motor regression.

Mitochondrial DNA Depletion Syndrome-3 (MTSPS3)

[0290] Mitochondrial DNA Depletion Syndrome-3, also known as hepatocerebral syndrome, is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the nuclear-encoded DGUOK gene. MTSPS3 is characterized by onset in infancy of progressive liver failure and neurologic abnormalities, hypoglycemia, and increased lactate in body fluids. Affected tissues show both decreased activity of the mtDNA-encoded respiratory chain complexes (I, III, IV, and V) and mtDNA depletion. Clinical symptoms include weakness, hypotonia, lactic acidosis, elevated serum creatine kinase, renal dysfunction, and ragged red fibers.

Mitochondrial Encephalopathy Lactic Acidosis Stroke (MELAS)

[0291] MELAS Syndrome, comprising mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, is a genetically heterogeneous mitochondrial disorder with a variable clinical phenotype. MELAS Syndrome can be caused by mutations in several genes, including POLG, MTTL1, MTTQ, MTTH, MTTK, MTTF, MTTC, MTTS1, MTTV, MTTQ, MTND1, MTND3, MTND5, MTND6, MTCOI, cytochrome b, and MTTS2, with mutations in MTTL1 accounting for the majority of MELAS cases. In particular, it is estimated that approximately 80% of MELAS patients have an A3243G point mutation in the

MTTL1 gene. The disorder is accompanied by features of central nervous system involvement, including seizures, hemiparesis, hemianopsia, cortical blindness, and episodic vomiting. Primary causes of death are cardiopulmonary failure, status epilepticus, and pulmonary disease.

[0292] Clinical symptoms include distal arthrogryposis, headache and vomiting, sensorineural hearing loss, seizures, loss of consciousness, dementia, mental retardation, focal events (strokes), cortical visual defects, hemiplegia, neuronal hyperexcitability, basal ganglia calcifications, weakness, exercise intolerance, ptosis, external ophthalmoplegia, gait disorder, paresthesias and numbness, reduced tendon reflexes, sensory neuropathy, chorea, Parkinsonism, ataxia, pigmentary retinopathy, macular dystrophy, optic atrophy, visual field defects, hypertelorism, hypertrophic cardiomyopathy, left ventricular noncompaction, conduction defects (such as Wolff-Parkinson-White), hypertension, short stature, maternally inherited diabetes (MIDD), pancreatitis, constipation, diarrhea, intestinal pseudoobstruction (ileus), nausea, dysphagia, abdominal pain, epigastralgia, sialoadenitis focal segmental glomerulosclerosis, renal cysts, tubular dysfunction, nephrotic syndrome, multihormonal hypopituitarism, Hashimoto thyroiditis, goiter, Hypoparathyroidism, Addison's disease, ovarian failure, miscarriage, lipoma, Atopic dermatitis, local melanoderma, asymmetric vascular dilatation, lactic acidosis, white matter lesions, respiratory chain dysfunction, ragged red fibers, cortical atrophy, focal necrosis, Purkinje dendrite cactus formations with increased mitochondria, and mitochondrial capillary angiopathy.

[0293] Other MTTF disorders include myoglobinuria, MERRF, camptocormia, seizures, and ataxia.

<u>Myopathy and External Ophthalmoplegia; Neuropathy; Gastro-Intestinal; Encephalopathy (MNGIE)</u>

[0294] Mitochondrial DNA Depletion Syndrome-1 (MTDPS1), which manifests as a neurogastrointestinal encephalopathy (MNGIE), is caused by homozygous or compound heterozygous mutations in the nuclear-encoded thymidine phosphorylase gene (TYMP). TYMP catalyzes phosphorolysis of thymidine to thymine and deoxyribose 1-phosphate, and plays a role in homeostasis of cellular nucleotide pools. Mitochondrial DNA Depletion Syndrome-1 (MTDPS1) is an autosomal recessive progressive multisystem disorder clinically characterized by onset between the second and fifth decades of life of ptosis, progressive external ophthalmoplegia (PEO), retinal degeneration, optic atrophy, gastrointestinal

dysmotility (often pseudoobstruction, gastroparesis, obstipation, malabsorption, diarrhea, abdominal pain & cramps, nausea & vomiting), borborygmi, early satiety, cachexia, thin body habitus, short stature, diffuse leukoencephalopathy, myopathy (proximal weakness, exercise intolerance), peripheral neuropathy (sensory loss/pain/ataxia, weakness, tendon reflexes absent, axonal loss, demyelination), hearing loss, cognitive impairment or dementia, seizures, headaches, and mitochondrial dysfunction. Mitochondrial DNA abnormalities can include depletion, deletion, and point mutations. MNGIE usually presents at < 20 years of age. Additional symptoms include incomplete right bundle branch block (cardiac defect), diabetes or glucose intolerance, amylase increase, exocrine insufficiency, neoplasms, lactic acidosis, elevated plasma thymidine levels, elevated plasma deoxyuridine & deoxythymidine levels, tetany, cardiac arrhythmia, high CSF protein, brain atrophy, mitochondrial changes in muscle fibers and neurogenic changes.

[0295] Partial loss of thymidine phosphorylase activity can result in a variant MNGIE disorder that manifests around the 5th decade of life. Clinical symptoms include ophthalmoplegia, ptosis, gastrointestinal features, and axon loss with or without demyelination.

[0296] Mitochondrial DNA Depletion Syndrome-4B (MTDPS4B), which manifests as a neurogastrointestinal encephalopathy (MNGIE), is caused by compound heterozygous mutations in the nuclear-encoded POLG gene. Mitochondrial DNA Depletion Syndrome-4B is an autosomal recessive progressive multisystem disorder clinically characterized by chronic gastrointestinal dysmotility and pseudoobstruction, cachexia, progressive external ophthalmoplegia (PEO), axonal sensory ataxic neuropathy, and muscle weakness.

[0297] Another MNGIE variant is MNGIM Syndrome without encephalopathy, which is not associated with mutations in thymidine phosphorylase or dNT-2. Clinical features include gastrointestinal malabsorption, diarrhea, borborygmi, abdominal pain, GI pseudo-obstruction, weight loss, ophthalmoplegia, ptosis, weakness, cachexia, polyneuropathy (pain, gait disorder, sensory ataxia, axonal loss), high CSF protein, ragged red fibers, and reduced Complex I–IV activities.

[0298] Mutations in MTTW can manifest as a neurogastrointestinal encephalopathy (MNGIE). Patients present at 1 year of age with recurrent vomiting and failure to thrive. Leg discomfort, cognitive regression, seizures, muscle wasting, and incontinence manifest

later during childhood. Other features include sensorineural deafness, ptosis, ophthalmoplegia, pigmentary retinopathy, constricted visual fields, short stature, feeding difficulties with constipation, colitis and diarrhea, high lactate levels in blood and CSF, brain atrophy, and periventricular white matter changes. Muscle biopsies show COX-negative fibers and low activity of Complexes I and IV.

[0299] Mutations in MTTV can manifest as a neurogastrointestinal encephalopathy (MNGIE). Age of onset is usually during early childhood. Clinical symptoms include cachexia, headache, gastrointestinal motility problems (Ileus, Abdominal pain; Megacolon), hearing loss, developmental delay, high serum lactate, COX- negative fibers and low activity of complexes I and IV. Disruption of MTTV function can also lead to Ataxia, Seizures & Hearing loss, and Learning difficulties, Hemiplegia & Movement disorder.

Menkes Disease, Occipital Horn Syndrome and X-linked Distal Spinal Muscular Atrophy-3

[0300] Menkes disease is an X-linked recessive disorder characterized by generalized copper deficiency and is caused by mutations in the ATP7A gene. Menkes disease usually manifests at birth and its clinical features result from the dysfunction of several copper-dependent enzymes. Clinical symptoms include seizures, pili torti, bladder diverticula, skin laxity, occipital exostoses, chronic diarrhea, acute onset of severe intra-abdominal bleeding, hemorrhagic shock, multiple fractures, hypoglycemia, hypothermia, feeding difficulties, hair with an abnormal texture, low serum copper and ceruloplasmin levels, subdural hematomas, high arched palate, wormian bones in the lambdoid suture of the occipital region, developmental delay, and speech loss.

[0301] Occipital Horn Syndrome (OHS) is caused by mutations in the gene encoding $Cu(^{2+})$-transporting ATPase, alpha polypeptide (ATP7A). Occipital Horn Syndrome is a rare connective tissue disorder characterized by hyperelastic and bruisable skin, hernias, bladder diverticula, hyperextensible joints, varicosities, and multiple skeletal abnormalities. The disorder is sometimes accompanied by mild neurologic impairment, and bony abnormalities of the occiput are a common feature, giving rise to the name. Clinical features include severe congenital cutis laxa, extremely loose skin, with truncal folds and sagging facial skin, pectus excavatum, craniotabes, stridor, sparse coarse hair, fragmented elastin fibers, and low serum copper.

[0302] X-linked distal spinal muscular atrophy-3 (SMAX3) is caused by mutations in the copper transport gene ATP7A and is characterized by spinal muscular atrophy affecting both the upper and lower limbs. Onset ranges from 1 to 10 years of age. Clinical symptoms include foot deformity (pes cavus or pes varus), gait instability, distal motor weakness and atrophy.

Methemoglobinemia

[0303] Methemoglobinemia is an autosomal recessive disorder characterized by decreased oxygen carrying capacity of the blood, resulting in cyanosis and hypoxia. Methemoglobinemia is associated with mutations in the CYB5R3 gene, which encodes cytochrome b5 reductase-3, an enzyme localized to the mitochondrial outer membrane where it catalyzes the transfer of reducing equivalents from NADH to cytochrome b5. There are two types of methemoglobin reductase deficiency. In type I, the defect affects the soluble isoform of CYB5R3, which is expressed in erythrocytes and functions to reduce methemoglobin to hemoglobin. In type II, the defect affects both soluble and microsomal isoforms of the enzyme, which play a role in physiologic processes including cholesterol biosynthesis and fatty acid elongation and desaturation. Type II methemoglobinemia is associated with mental deficiency and other neurologic symptoms.

Myoclonic Epilepsy Ragged Red Fibers (MERRF)

[0304] MERRF Syndrome represents a maternally-inherited myopathy that can be produced by mutations in more than 1 mitochondrial gene, *e.g.*, MTTK, MTTL1, MTTH, MTTS1, MTTS2, MTTF *etc.* Features of the MERRF Syndrome have also been associated with mutations in the MTND5 gene.

[0305] Clinical features include myoclonus, epilepsy, cardiomyopathy, ataxia, gait disorder, dementia, optic atrophy, distal sensory loss, hearing loss, weakness, muscle pain, cramps, fatigue, short stature, lipomata, ragged red fibers, vacuoles in small fibers, and reduced Complex I, III and IV activity.

[0306] Other MTTK syndromes include cardiomyopathy, progressive external ophthalmoplegia with myoclonus, deafness and diabetes (DD), multiple symmetric lipomatosis, Leigh Syndrome, MELAS, MNGIE, Myopathy with Episodic high Creatine Kinase (MIMECK), Parkinson syndrome neuropathy and myopathy. Clinical features of MIMECK include weakness, dysphagia, and episodic myalgias.

[0307] Other MTTS1 disorders include MELAS, Epilepsia Partialis Continua, HAM Syndrome, myopathy , encephalopathy with cytochrome c oxidase deficiency, Myoclonus, epilepsy, cerebellar ataxia & progressive hearing loss, exercise intolerance, keratoderma, palmoplantar, with deafness, and sensorineural hearing loss.

[0308] Mutations in MTTP can also result in myoclonic epilepsy, myopathy, sensorineural deafness, cerebellar ataxia, and pigmentary retinopathy.

Myoglobinuria

[0309] Myoglobinuria can arise as a result of malignant hyperthermia syndromes, glycogen metabolic disorders, fatty acid oxidation and lipid metabolism disorders, mitochondrial disorders, certain drugs and toxins, hypokalemic myopathy and rhabdomyolysis, muscle trauma, ischemia, infections, or immune myopathy. Other disorders associated with occasional myoglobinuria include Brody myopathy, cylindrical spiral (myofilamentous) myopathy, familial recurrent rhabdomyolysis, fingerprint body disease, G6PDH deficiency, hypokalemic periodic paralysis, Marinesco-Sjögren Syndrome, myoadenylate deaminase deficiency, myotonias, multicore disease, Native American Myopathy, Schwartz-Jampel Syndrome (chondrodystrophic myotonia), sickle cell anemia, and several muscular dystrophies including Duchenne and Becker muscular dystrophy, Miyoshi myopathy, sacroglycanopathies, limb-girdle muscular dystrophy-dystroglycanopathy type C5, and limb-girdle muscular dystrophy-2L.

1. Malignant Hyperthermia Syndromes

[0310] Malignant hyperthermia syndromes leading to myoglobinuria can include central core disease, King-Denborough Syndrome, also known as malignant hyperthermia susceptibility 1 (MHS1), malignant hyperthermia susceptibility 2 (MHS2) malignant hyperthermia susceptibility 3 (MHS3), malignant hyperthermia susceptibility 4 (MHS4), malignant hyperthermia susceptibility 5 (MHS5), malignant hyperthermia susceptibility 6 (MHS6), and other disorders associated with malignant hyperthermia susceptibility, including Duchenne and Becker muscular dystrophies, myotonic dystrophy, myotonia congenital, Schwartz-Jampel Syndrome, and Satoyoshi Syndrome.

2. Glycogen Metabolic Disorders

[0311] Glycogen metabolic disorders leading to myoglobinuria can include McArdle disease, also known as glycogen storage disease type V (GSD5), Tarui disease, also known as glycogen storage disease VII (GSD7), and other glycogenoses, including aldolase A deficiency, also known as glycogen storage disease XII (GSD12), lactate dehydrogenase A deficiency, also known as glycogen storage disease XI (GSD11), phosphoglycerate kinase-1 deficiency, phosphoglycerate mutase deficiency, also known as glycogen storage disease X (GSD10), phosphorylase kinase deficiency of liver and muscle, also known as glycogen storage disease IXb (GSD9B), Forbes disease, also known as glycogen storage disease III (GSD3) or glycogen debrancher deficiency, and β-enolase deficiency, also known as glycogen storage disease XIII (GSD13).

3. Fatty Acid Oxidation and Lipid Metabolism Disorders

[0312] Fatty acid oxidation and lipid metabolism disorders leading to myoglobinuria can include carnitine palmitoyltransferase II (CPT II) deficiency, acyl-CoA dehydrogenase deficiencies, α-methylacyl-CoA racemase (AMACR) deficiency, electron transfer flavoprotein disorders, ketoacyl CoA thiolase deficiency, recurrent acute myoglobinuria, also known as recurrent rhabdomyolysis in childhood, and trifunctional enzyme deficiency, also known as long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (LCHAD) deficiency.

[0313] Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is a rare autosomal recessive condition that prevents the body from converting certain fats to energy, particularly during periods without food (fasting). Mutations in the HADHA gene cause LCHAD deficiency. Signs and symptoms of LCHAD deficiency typically appear during infancy or early childhood and can include feeding difficulties, lack of energy (lethargy), low blood sugar (hypoglycemia), weak muscle tone (hypotonia), liver problems, and abnormalities in the light-sensitive tissue at the back of the eye (retina). Later in childhood, people with this condition may experience muscle pain, breakdown of muscle tissue, and a loss of sensation in their arms and legs (peripheral neuropathy). Individuals with LCHAD deficiency are also at risk for serious heart problems, breathing difficulties, coma, and sudden death. Problems related to LCHAD deficiency can be triggered by periods of fasting or by illnesses such as viral infections.

[0314] Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is an autosomal recessive condition caused by mutations in the ACADM gene. Signs and symptoms of MCAD deficiency typically appear during infancy or early childhood and can include vomiting, lack of energy (lethargy), and low blood sugar (hypoglycemia). In rare cases, symptoms of this disorder first appear during adulthood. People with MCAD deficiency are at risk for serious complications such as seizures, breathing difficulties, liver problems, brain damage, coma, and sudden death.

[0315] Long-chain acyl-CoA dehydrogenase (LCAD) deficiency is caused by mutations in the ACADL gene. Subjects with LCAD can present with SIDS, hypoglycemia, hepatomegaly, myopathy, Reye syndrome, and cardiomyopathy. The plasma acylcarnitine profile exhibits elevated long chain acyl-carnitine esters. Urine organic acids typically show elevations of dicarboxylic acids.

4. Mitochondrial Disorders

[0316] Mitochondrial disorders leading to myoglobinuria can include cytochrome c oxidase (COX) deficiencies, cytochrome b deficiency, mitochondrial myopathies, including coenzyme Q10 deficiency, myopathy with lactic acidosis, also known as Swedish type myopathy with exercise intolerance, dihydrolipoamide dehydrogenase (DLD) deficiency, mutations in the DGUOK gene, which encodes mitochondrial deoxyguanosine kinase, and iron-sulfur complex disorders, including those associated with mutations in one or more of the following genes: ISCU, FDX1L, NFU1, BOLA3, NUBPL, IBA57, LYRM4, and LYRM7.

5. Medication-, Drug- or Toxin-induced Myoglobinuria

[0317] Certain medications such as amiodarone, arsenic trioxide, emetine, ε-amino caproic acid, lipid lowering agents such as clofibrate and statins, isoniazid, lamotrigrine, antipsychotics, nicotinic acid, pentamidine, propofol, proton pump inhibitors, selective serotonin reuptake inhibitors, irinotican, temazepam, valproate, vasopressin, and zidovudine are associated with the development of myoglobinuria. Drugs and toxins associated with the development of myoglobinuria include cocaine, heroin, snake venom, insect venom, blowpipe dart poison, and drugs and toxins that produce muscle overactivity, including amphetamines, hemlock, loxpapine, LSD, mercuric chloride, phencyclidine, strychnine, tetanus toxin, and terbutaline. The ingestion of certain toxins including ethanol, monensin,

chromium picolinate, methylenedioxypyrovalerone, mephedrone, phencyclidine, and those present in Buffalo fish, burbot, mushrooms (Amanita phalloides, Trichloma equestre), kidney beans, and peanut oil may also cause myoglobinuria.

[0318] Other drugs and toxins associated with the development of myoglobinuria include acetaminophen, amoxapine, anticholinergics, azathioprine, baclofen, barbiturates, benzodiazepines, butyrophenones, caffeine, chloral hydrate, chlorpromazine, colchicine, corticosteroids, daptomycin, diphenhydramine, doxylamine, ephedra, fenfluramine, glutethimide, hydroxyzine, ketamine, lysergic acid diethylamide, methanol, minocycline, morphine, phencyclidine, phenothiazines, phentermine phenytoin, quinolones, salicylate, serotonin antagonists, succinylcholine, sunitinib sympathomimetics, theophylline, trimethoprim-sulfamethoxazole, and vincristine.

6. Hypokalemic Myopathy and Rhabdomyolysis

[0319] Hypokalemic myopathy and rhabdomyolysis can be of a pharmacologic or toxic origin. Hypokalemic myopathy and rhabdomyolysis having a pharmacologic origin is associated with diuretics and laxatives, such as thiazides, amphotericin, lithium, gossypol, methylxanthines, and laxative abuse. Toxins associated with the development of hypokalemic myopathy and rhabdomyolysis include glycyrrhizic acid, glycyrrhetinic acid, barium, ethanol, cottonseed oil, and volatile substances, such as toluene.

7. Muscle trauma

[0320] Muscle trauma leading to myoglobinuria can be acute, such as that associated with physical trauma, or chronic, such as that associated with alcohol, opiates, and sedatives. Muscle trauma can also be caused by overactivity due to exercise, drugs and toxins producing muscle overactivity, hyperthermia, or seizures. Muscle trauma can also be caused by compartment syndromes and temperature alterations associated with heat stroke, malignant hyperthermia, neuroleptic malignant syndrome, burns, or hypothermia.

8. Ischemia

[0321] Ischemia leading to myoglobinuria can be caused by vascular occlusion, hemangioma steal syndrome, sickle cell trait, cocaine, calciphylaxis, compartment syndrome, carbon monoxide exposure, or cyanide poisoning.

9. Infections

[0322] Infections associated with myoglobinuria include viral infections such as influenza A and B, coxsackie virus, herpes, adenovirus, and HIV-1; bacterial infections including Streptococci, Salmonella, Staphylococci, typhoid fever, Legionella, Clostridia, and E. coli; Mediterranean tick typhus; tick-borne infections such as ehrlichiosis, anaplasmosis, baesiosis, Lyme disease, and Rocky Mountain spotted fever; and hyperthermia-related infections.

10. Immune myopathy

[0323] Immune myopathies associated with myoglobinuria include polymyositis and dermatomyositis.

Myopathy, Lactic Acidosis and Sideroblastic Anemia (MLASA)

[0324] Myopathy, lactic acidosis, and sideroblastic anemia (MLASA) is a rare autosomal recessive oxidative phosphorylation disorder specific to skeletal muscle and bone marrow. Myopathy, lactic acidosis, and sideroblastic anemia-1 (MLASA1) can be caused by homozygous mutations in the PUS1, which converts uridine into pseudouridine after the nucleotide has been incorporated into RNA. Pseudouridine may have a functional role in tRNAs and may assist in the peptidyl transfer reaction of rRNAs. MLASA1 usually presents in children and teens and is characterized by progressive weakness, exercise intolerance, fatigue, nausea and vomiting, ptosis, short stature, sideroblastic anemia, lactic acidosis and reduced mitochondrial oxidative enzyme activities.

[0325] Myopathy, lactic acidosis, and sideroblastic anemia-2 (MLASA2) is an autosomal recessive disorder of the mitochondrial respiratory chain that is caused by homozygous mutations in the aminoacyl-tRNA synthetase gene YARS2. The disorder shows marked phenotypic variability: some patients have a severe multisystem disorder from infancy, including cardiomyopathy and respiratory insufficiency resulting in early death, whereas others present in the second or third decade of life with sideroblastic anemia and mild muscle weakness. Additional clinical features include dysphagia, weakness, exercise intolerance, short stature, high serum lactate, reduced COX activity, and reduced Complex I, III and IV activity.

Infantile Mitochondrial Myopathy due to Reversible COX Deficiency (MMIT)

[0326] Infantile mitochondrial myopathy due to reversible COX deficiency is a rare mitochondrial disorder characterized by onset in infancy of severe hypotonia and generalized muscle weakness associated with lactic acidosis, but is distinguished from other mitochondrial disorders in that affected individuals recover spontaneously after 1 year of age. MMIT is caused by mutations in the MTTE gene, which is encoded by the mitochondrial genome.

[0327] Clinical features include muscle weakness, hypotonia, respiratory failure, dysphagia, ophthalmoplegia, macroglossia, neuropathy, seizures, encephalopathy, hepatomegaly, pneumonia, lactic acidosis, delayed myelination, high serum creatine kinase, ragged red fibers, reduced COX activity, reduced Complex I activity, increased lipid or glycogen in muscle fibers, muscle degeneration, inflammation, lipid droplets in fibers, and mtDNA reduction.

[0328] Variant MTTE syndromes include mitochondrial myopathy with diabetes mellitus, diabetes-deafness syndrome, LHON, Mitochondrial myopathy with respiratory failure, MELAS/LHON/DEAF, progressive encephalopathy, encephalomyopathy with retinopathy, leukoencephalopathy and exercise intolerance.

[0329] Additionally, certain MTTE mutations can lead to at least one or more symptoms such as myopathy, ataxia, lactic acidosis, high serum creatine kinase, SDH+ & COX negative muscle fibers, reduced Complexes I, III & IV activity, retinopathy, severe myopathy with respiratory failure, ptosis, PEO, pigmentary retinopathy, migraines, life-long exercise intolerance and leukodystrophy.

Specific Sporadic Mitochondrial Myopathy Syndromes

1. Myopathy, Exercise Intolerance, Encephalopathy, Lactic acidemia

[0330] Cytochrome c oxidase subunit III (COIII or MTCO3) is 1 of 3 mitochondrial DNA (mtDNA) encoded subunits (MTCO1, MTCO2, MTCO3) of respiratory Complex IV. Complex IV is located within the mitochondrial inner membrane and is the third and final enzyme of the electron transport chain of mitochondrial oxidative phosphorylation. It collects electrons from ferrocytochrome c (reduced cytochrome c) and transfers then to oxygen to give water. The energy released is to transport protons across the mitochondrial

inner membrane. Complex IV is composed of 13 polypeptides. Subunits I, II, and III (MTCO1, MTCO2, MTCO3) are encoded by the mtDNA while subunits VI, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc, and VIII are nuclear encoded. Subunits VIa, VIIa, and VIII have systemic as well as heart muscle isoforms.

[0331] Mutations in MTCO3 can result in Myopathy, Exercise intolerance, Encephalopathy, Lactic acidemia Syndrome, which usually manifests between 4 to 20 years of age. Clinical symptoms include weakness, myalgia, fatigue, myoglobinuria, encephalopathy, migraine, spastic paraparesis, mental retardation, ophthalmoplegia, high serum lactate, COX deficiency, SDH positive muscle fibers, and lipid accumulation in type I fibers.

[0332] Mutations in MTCO3 can also result in isolated myopathy (characterized by weakness, COX deficiency, persistent ragged red fibers), myoglobinuria, maternally inherited myopathies, MELAS-like disorder, Leigh-like disorder, and nonarteritic ischemic optic neuropathy (NAION)-Myoclonic epilepsy.

2. <u>Myoglobinuria & Exercise Intolerance</u>

[0333] Mutations in MTCO1 can result in Myoglobinuria and exercise intolerance, which usually manifests at childhood. Clinical symptoms include exercise intolerance, myoglobinuria, COX deficiency, and defects in Complex I & III. Other MTCO1 syndromes include acquired sideroblastic anemia; Deafness, Ataxia, Blindness, Myopathy; epilepsy partialis continua; motor neuron disease; LHON; Myopathy, Cardiomyopathy, Stroke; MELAS-like Syndrome.

3. <u>Exercise Intolerance, Proximal Weakness ± Myoglobinuria</u>

[0334] Cytochrome b (MTCYB) is the only mitochondrial DNA (mtDNA) encoded subunit of respiratory Complex III (ubiquinol:ferrocytochrome c oxidoreductase, or cytochrome bc1, complex). Complex III is located within the mitochondrial inner membrane and is the second enzyme in the electron transport chain of mitochondrial oxidative phosphorylation. It catalyzes the transfer of electrons from ubiquinol (reduced Coenzyme Q10) to cytochrome c and utilizes the energy to translocate protons from inside the mitochondrial inner membrane to outside.

[0335] Disruption of MTCYB function can result in exercise intolerance, proximal weakness ± myoglobinuria syndrome, which manifests during childhood. Symptoms include sensation of cramps, myalgias or fatigue, weakness, myoglobinuria, septo-optic dysplasia, retardation, encephalopathy, seizures, high serum lactate, myopathy, deficient Complex III activity, and ragged red fibers.

[0336] Mutations in MTCYB can lead to Encephalopathy & Seizures Syndrome, which usually presents at 9 to 13 years of age. Clinical features include exercise intolerance, lactic acidosis, encephalopathy, poor balance, seizures, visual hallucinations, depression, emotional lability, and ragged red fibers.

[0337] Other variant disorders caused by mutations in MTCYB include septo-optic dysplasia (characterized by mental retardation, delayed walking, exercise intolerance, retinitis pigmentosa, optic atrophy, hypertrophic cardiomyopathy, Wolff-Parkinson-White, lactic acidosis, and cerebellar hypoplasia), Familial Myalgia Syndrome, Exercise intolerance, LHON, colon cancer, LVNC, MELAS, Parkinsonism, obesity, and Migraine, Epilepsy, Polyneuropathy, Stroke-like episodes.

4. <u>Exercise Intolerance ± Mild Weakness</u>

[0338] Mutations in several mitochondrial genes such as MTTW, Cytochrome b (Complex III), MTND1 (Complex I), MTND2 (Complex I), and MTND4 (Complex I) can cause exercise intolerance with or without mild weakness, which manifests at childhood. Additional clinical features include dyspnea, tachycardia, high serum lactate, and ragged red fibers.

5. <u>Myopathy ± Exercise Intolerance, Growth or CNS disorder</u>

[0339] Mutations in MTTM can result in Myopathy with or without Exercise intolerance, Growth or CNS disorders, which usually manifests at 10 to 56 years of age. Clinical symptoms include myopathy, proximal and distal weakness, exercise intolerance, muscle atrophy, ptosis, reduced tendon reflexes, growth retardation, mental retardation, lactic acidosis, high serum Creatine Kinase, ragged red fibers, COX deficiency, muscular dystrophy, cortical atrophy, and myelomalacia. Other variant MTTM syndromes include Exercise intolerance, Autoimmune polyendocrinopathy and Lactic acidosis.

6. Myopathy with Episodic High Creatine Kinase (MIMECK)

[0340] Mutations in MTTK can result in Myopathy with Episodic high Creatine Kinase (MIMECK), which usually manifests between the ages of 15 to 69 years. Clinical features include weakness, dysphagia, myalgias, and episodic high serum Creatine Kinase.

Maternally-Inherited Mitochondrial Myopathies

[0341] Maternally-inherited mitochondrial myopathies can be caused by mutations in Cytochrome c Oxidase, Subunit II (COX II or MTCO2). Myopathy usually manifests in children and teens. Clinical features include weakness, fatigue and exercise intolerance, rhabdomyolysis, ataxia, retinopathy, optic atrophy, reduction in COX activity, lipid in type I fibers, cataracts, hearing loss, cardiac arrhythmia, depression, short stature, lactic acidosis, elevated serum or CSF lactate and mitochondrial proliferation.

[0342] Mutations in MTTS1 can cause Myopathy, Deafness & CNS disorders that usually manifest at 8 years of age. Clinical features include weakness or contractures, fatigue, sensorineural deafness, ataxia, cognitive impairment, optic atrophy, axonal sensory neuropathy, high serum and CSF lactate, mitochondrial proliferation, COX- fibers, and reduced Complex I & IV activity.

[0343] Mutations in MTTW can cause Myopathy, Ptosis & Dysphonia, which usually manifests at 50 years of age. Clinical features include ptosis, weakness, fatigue, SDH+ and COX negative muscle fibers, and cytochrome c oxidase reduction.

[0344] Mutations in MTTE can cause Myopathy, Diabetes & CNS disorders, which usually manifest in teens or adults. Clinical features include fatigue, weakness, orbicularis oculi, respiratory failure, FSH dystrophy, fatigue, diabetes, polyneuropathy, cerebellar ataxia, nystagmus, congenital encephalopathy, endomysial fibrosis, mitochondrial proliferation, COX negative muscle fibers, reduced Complex I & IV activity, and focal COX reductions in cardiac muscle.

Autosomal Recessive Mitochondrial Myopathies

1. Myopathy with Lactic Acidosis

[0345] Myopathy with lactic acidosis, also known as Swedish type myopathy with exercise intolerance, is caused by homozygous or compound heterozygous mutations in the ISCU

gene, encoding the iron-sulfur cluster scaffold protein, on chromosome 12q24. Hereditary myopathy with lactic acidosis is an autosomal recessive muscular disorder characterized by childhood onset of exercise intolerance with muscle tenderness, cramping, dyspnea, and palpitations. Clinical features include fatigue, shortness of breath, tachycardia, weakness, lactic acidosis, rhabdomyolysis, muscle swelling, myalgias, cardiac hypertrophy, SDH deficiency, abnormality of muscle mitochondrial iron-sulfur cluster-containing proteins, high serum lactate, reduced COX expression, and mitochondrial inclusions. Disruption of ISCU function can also result in Myopathy with Myoglobinuria.

[0346] Iron-sulfur cluster disorders can also arise as a result of mutations in FDX1L, Glutaredoxin 5, NFU1, BOLA3, NUBPL, IBA57, LYRM4 and LYRM7.

2. Myopathy + Rhabdomyolysis

[0347] Myopathy with rhabdomyolysis is caused by mutations in Ferredoxin 1-like protein (FDX1L), which plays a role in Fe-S cluster biogenesis. Clinical symptoms include weakness, dyspnea, myoglobinuria, fatigue, reduced Complex I, II & III activities, reduced aconitase activity, high citrate synthase, high lactate, 3-methyl glutaconic acid, ketones & Krebs cycle metabolites.

3. Myopathy + Cataracts & Combined Respiratory Chain Defects

[0348] Myopathy with cataract and combined respiratory chain deficiency can be caused by mutations in the GFER gene, which plays a role in the mitochondrial disulfide relay system. Clinical features include reduced Complex I, II, and IV activity, accumulation of multiple mtDNA deletions, cataracts, hypotonia, developmental delay, muscle smallness, reduced tendon reflexes, sensorineural hearing loss, SDH+ and COX negative muscle fibers, high serum lactate, low serum ferritin, and high serum amylase.

4. Myopathy with Abnormal Mitochondrial Translation

[0349] Myopathy with abnormal mitochondrial translation is an autosomal disorder that manifests during childhood. Clinical symptoms include weakness, fatiguability, hypotonia, ptosis, ophthalmoplegia, short stature, sideroblastic anemia, mitochondrial proliferation in muscle fibers, reduced COX activity, reduced Complex I, II, III and IV activity, and mitochondrial translation defects.

5. Fatigue & Exercise Intolerance

[0350] Fatigue Syndrome is caused by homozygous or compound heterozygous mutations in the ACAD9 gene, which encodes a protein that catalyzes the initial rate-limiting step in β-oxidation of fatty acyl-CoA. ACAD9 deficiency is an autosomal recessive multisystemic disorder characterized by infantile onset of acute metabolic acidosis, hypertrophic cardiomyopathy, and muscle weakness associated with a deficiency of mitochondrial complex I activity in muscle, liver, and fibroblasts. Clinical features include exercise intolerance, urge to vomit, sense of mental slowness, reduced Complex I activity, high serum lactate. Episodic hepatic dysfunction is also present in some variants of the syndrome.

6. Myopathy with Extrapyramidal Movement Disorders (MPXPS)

[0351] Myopathy with extrapyramidal signs is an autosomal recessive disorder characterized by early childhood onset of proximal muscle weakness and learning disabilities. While the muscle weakness is static, most patients develop progressive extrapyramidal signs that may become disabling. Myopathy with extrapyramidal signs (MPXPS) is caused by homozygous mutations in the MICU1 gene, which plays a role in mitochondrial Ca^{2+} uptake. Clinical features include chorea, tremor, dystonia, orofacial dyskinesia, ataxia, microcephaly, ophthalmoplegia, ptosis, optic atrophy, and peripheral neuropathy.

7. Glutaric aciduria II (MADD)

[0352] MADD, also known as glutaric acidemia II or glutaric aciduria II, can be caused by mutations in at least 3 different genes: ETFA, ETFB, and ETFDH. These genes are all involved in electron transfer in the mitochondrial respiratory chain. The disorders resulting from defects in these 3 genes are referred to as glutaric acidemia IIA, IIB, and IIC, respectively, although there appears to be no difference in the clinical phenotypes.

[0353] Glutaric aciduria II (GA II) is an autosomal recessively inherited disorder of fatty acid, amino acid, and choline metabolism. It differs from GA I in that multiple acyl-CoA dehydrogenase deficiencies result in large excretion not only of glutaric acid, but also of lactic, ethylmalonic, butyric, isobutyric, 2-methyl-butyric, and isovaleric acids. GA II results from deficiency of any 1 of 3 molecules: the alpha (ETFA) and beta (ETFB) subunits of electron transfer flavoprotein, and electron transfer flavoprotein dehydrogenase (ETFDH).

[0354] The heterogeneous clinical features of patients with MADD fall into 3 classes: a neonatal-onset form with congenital anomalies (type I), a neonatal-onset form without congenital anomalies (type II), and a late-onset form (type III). The neonatal-onset forms are usually fatal and are characterized by severe nonketotic hypoglycemia, metabolic acidosis, multisystem involvement, and excretion of large amounts of fatty acid- and amino acid-derived metabolites. Symptoms and age at presentation of late-onset MADD are highly variable and characterized by recurrent episodes of lethargy, vomiting, hypoglycemia, metabolic acidosis, and hepatomegaly often preceded by metabolic stress. Muscle involvement in the form of pain, weakness, and lipid storage myopathy also occurs. The organic aciduria in patients with the late-onset form of MADD is often intermittent and only evident during periods of illness or catabolic stress.

8. Coenzyme Q10 Deficiency

[0355] Primary CoQ10 deficiency is a rare, clinically heterogeneous autosomal recessive disorder caused by mutation in any of the genes encoding proteins directly involved in the synthesis of coenzyme Q. Coenzyme Q10 (CoQ10), or ubiquinone, is a mobile lipophilic electron carrier critical for electron transfer by the mitochondrial inner membrane respiratory chain.

[0356] The disorder has been associated with 5 major phenotypes, but the molecular basis has not been determined in most patients with the disorder and there are no clear genotype/phenotype correlations. The phenotypes include an encephalomyopathic form with seizures and ataxia; a multisystem infantile form with encephalopathy, cardiomyopathy and renal failure; a predominantly cerebellar form with ataxia and cerebellar atrophy; Leigh Syndrome with growth retardation; and an isolated myopathic form.

[0357] Autosomal recessive forms of the disorder include COQ10D1, caused by mutations in COQ2; COQ10D2, caused by mutations in the PDSS1 gene; COQ10D3, caused by mutations in the PDSS2 gene; COQ10D4, caused by mutations in the COQ8 gene (ADCK3); COQ10D5, caused by mutations in the COQ9 gene; and COQ10D6, caused by mutations in the COQ6 gene.

[0358] Secondary CoQ10 deficiency has been reported in association with glutaric aciduria type IIC (MADD), caused by mutation in the ETFDH gene, and with ataxia-oculomotor apraxia syndrome-1 (AOA1), caused by mutation in the APTX gene.

Autosomal Dominant Mitochondrial Myopathy

[0359] Dominant mutations in coiled-coil-helix-coiled-coil-helix domain-containing protein 10 (CHCHD10) can lead to mitochondrial myopathy with exercise intolerance, which usually manifests within the first decade of life. Clinical features include exercise intolerance, weakness in legs, arms, and face, restrictive deficits in pulmonary function, short stature, high serum lactate, high serum pyruvate, ragged red fibers, reduced cytochrome c oxidase (Complex IV) activity, and reduced succinate cytochrome c reductase (Complex II & III) activity.

[0360] Other examples of autosomal dominant mitochondrial myopathies include myopathy with focal depletion of mitochondria, mitochondrial DNA breakage syndrome (PEO + Myopathy), LGMD1H, and lipid type mitochondrial myopathy.

Multiple Symmetric Lipomatosis (Madelung Syndrome)

[0361] Multiple symmetric lipomatosis (MSL) is a rare disorder characterized by the growth of uncapsulated masses of adipose tissue. It is associated with high ethanol intake and may be complicated by somatic and autonomic neuropathy and by the infiltration of the adipose tissue at the mediastinal level. MSL can arise as a result of an autosomal dominant, mitochondrial, or sporadic mutation. Clinical features include multiple lipomas in the nape of the neck, supraclavicular and deltoid regions, full body lipomas, polyneuropathy, hyperuricemia, high triglycerides (VLDL, chylomicrons), high HDL, and ragged red fibers.

N-acetylglutamate Synthase Deficiency

[0362] N-acetylglutamate synthase (NAGS) deficiency is an inborn error of metabolism affecting ammonia detoxification in the urea cycle. N-acetylglutamate synthase is a mitochondrial enzyme that catalyzes the formation of N-acetylglutamate (NAG). NAG is an essential allosteric activator of carbamoylphosphate synthase 1 (CPS1), the first and rate-limiting enzyme in the urea cycle. Most NAGS genes contain a C-terminus transferase domain in which the catalytic activity resides and an N-terminus kinase domain where arginine binds. Because CPS1 is inactive without NAG, the urea cycle function can be severely affected resulting in fatal hyperammonemia in neonatal patients or at any later stage in life. Clinical manifestations of NAGS deficiency include poor feeding, vomiting, altered levels of consciousness, seizures, and coma.

Neoplasms

[0363] Mutations in certain nuclear encoded mitochondrial genes can give rise to neoplasms, such as paraganglionoma, leiomyomatosis, renal cell cancer, and B-cell lymphoma. Paragangliomas, also referred to as 'glomus body tumors,' are tumors derived from paraganglia located throughout the body. Nonchromaffin types primarily serve as chemoreceptors (hence, the tumor name 'chemodectomas') and are located in the head and neck region (*i.e.*, carotid body, jugular, vagal, and tympanic regions), whereas chromaffin types have endocrine activity, conventionally referred to as 'pheochromocytomas,' and are usually located below the head and neck (*i.e.*, adrenal medulla and pre- and paravertebral thoracoabdominal regions). PGL can manifest as nonchromaffin head and neck tumors only, adrenal and/or extraadrenal pheochromocytomas only, or a combination of the 2 types of tumors.

[0364] Familial paragangliomas-1 (PGL1) is caused by mutations in the SDHD gene, which encodes the small subunit of cytochrome B in succinate-ubiquinone oxidoreductase. PGL1 manifests as benign vascularized tumors in the head and neck.

[0365] Hereditary paragangliomas-2 (PGL2) is caused by mutations in the SDHAF2 gene, which encodes a protein necessary for flavination of SDHA. PGL1 manifests as tumors in the head and neck, and especially the carotid body.

[0366] Hereditary paragangliomas-3 (PGL3) is caused by heterozygous mutations in the SDHC gene, which encodes subunit C of the succinate dehydrogenase complex. PGL3 manifests as benign vascularized tumors in the head and neck.

[0367] Familial paragangliomas-4 (PGL4) is caused by heterozygous mutations in the SDHB gene, which encodes the iron sulfur subunit of succinate dehydrogenase. Clinical features include susceptibility to pheochromocytoma and paraganglioma, and Cowden-like Syndrome (CWD2).

[0368] Paragangliomas-5 (PGL5) can be caused by heterozygous mutations in the SDHA gene. Clinical features include hypertension and hyperadrenergic symptoms, such as dizziness, tachycardia, and sweating. Patients exhibit high concentrations of urinary normetanephrine, norepinephrine, and chromogranin A. Perturbations in SDHA can also lead to cardiomyopathy and mitochondrial respiratory chain complex 2 deficiency.

[0369] BCL2 is an integral inner mitochondrial membrane protein of relative molecular mass 25,000. Overexpression of BCL2 blocks the apoptotic death of a pro-B-lymphocyte cell line, and can result in B-cell lymphoma. Thus, BCL2 is unique among proto-oncogenes, being localized in mitochondria and interfering with programmed cell death independent of promoting cell division.

[0370] Heterozygous mutations in the fumarate hydratase gene can cause hereditary leiomyomatosis and renal cell cancer.

[0371] Susceptibility to the development of neuroblastoma-1 (NBLST1) and isolated pheochromocytoma is associated with mutations in the KIF1B gene, which encodes kinesin family member 1B. This protein is a member of the kinesin family of proteins that are essential for intracellular transport, including the transport of mitochondria. NBLST1 is common neoplasm of early childhood arising from embryonic cells that form the primitive neural crest and give rise to the adrenal medulla and the sympathetic nervous system. Pheochromocytoma is caused by a catecholamine-producing tumor of chromaffin tissue of the adrenal medulla or sympathetic paraganglia. The cardinal symptom, reflecting the increased secretion of epinephrine and norepinephrine, is hypertension, which may be persistent or intermittent.

Nephronophthisis

[0372] Nephronophthisis (NPHP), also known as nephronophthisis-like nephropathy 1, is an autosomal recessive cystic kidney disease characterized by the onset of end-stage renal failure in the first three decades of life. Features of NPHP include irregular tubular basement membrane, tubular cyst formation, and interstitial cell infiltration with fibrosis. The disorder is also frequently associated with extrarenal manifestations including liver fibrosis, retinal degeneration, and central nervous system abnormalities. Mutations in ten causative genes (NPHP1-NPHP9 and NPHP11), whose products localize to the primary cilia-centrosome complex, have been identified and are linked to the development of NPHP. In addition, homozygous frameshift and splice-site mutations in the X-prolyl aminopeptidase 3 (XPNPEP3) gene have been identified and are associated with the development of a nephronophthisis-like nephropathy. In contrast to all known NPHP proteins, XPNPEP3 localizes to mitochondria of renal cells. XPNPEP3 belongs to a family of X-pro-aminopeptidases that utilize a metal cofactor and remove the N-terminal amino acid from peptides with a proline residue in the penultimate position.

Neuropathy; Ataxia; Retinitis Pigmentosa (NARP)

[0373] NARP Syndrome is caused by mutations in the gene encoding subunit 6 of mitochondrial H(+)-ATPase (MTATP6) and usually presents at childhood (2nd decade) or adult stage. The MT-ATP6 protein forms one subunit of complex V (ATP synthase), which is responsible for the last step in ATP production. Mutations in MT-ATP6 alter the structure or function of ATP synthase, reducing the ability of mitochondria to produce ATP. Most individuals with NARP have a specific point mutation at nucleotide 8993, with a T8993G mutation causing more severe symptoms than a T8993C mutation. Some cases involve a G8989C point mutation.

[0374] Clinical features include sensory neuropathy, proximal and distal weakness, reduced tendon reflexes, retinitis pigmentosa, reduced night vision, Bull's eye maculopathy, pigment in posterior pole & mid-periphery, small retinal scars, vascular narrowing, central and paracentral scotomas, gait disorder, dysarthria, dementia, seizures, Tonic-clonic seizures, developmental delay, pyramidal signs, dystonia, hearing loss, cardiac hypertrophy, denervation, cerebral atrophy, cortical cerebellar atrophy, focal cystic necrosis, and rod or cone dysfunction.

[0375] Disruption of MTATP6 function can result in Distal Hereditary Motor Neuropathy (dHMN), which usually presents during the 1st or 2nd decade of life. Clinical features include gait disorder, weakness, sensory loss, brisk tendon reflexes, extensor plantar reflex, pes cavus, Kyphoscoliosis, motor axon loss, sensory axon loss, and reduced Complex V activity.

Ornithine Transcarbamylase Deficiency

[0376] Ornithine transcarbamylase (OTC) deficiency is an X-linked inborn error of metabolism of the urea cycle that causes hyperammonemia. Ornithine carbamoyltransferase is a nuclear-encoded mitochondrial matrix enzyme that catalyzes the second step of the urea cycle in mammals. OTC deficiency is associated with mutations in the OTC gene. OTC is the most common urea cycle defect and is characterized by the triad of hyperammonemia, encephalopathy, and respiratory alkalosis.

Paroxysmal Nonkinesigenic Dyskinesia

[0377] Paroxysmal nonkinesigenic dyskinesia (PNKD) is an autosomal dominant movement disorder characterized by sudden attacks of dystonia, chorea, and athetosis. PNKD is associated with mutations in the myofibrillogenesis regulator-1 gene (MR1). MR1 is transcribed into three alternatively spliced isoforms: long (MR-1L); medium (MR-1M); and small (MR-1S). The MR-1L and MR-1M isoforms are mitochondrial proteins imported into the organelle by a 39-amino acid, N-terminal mitochondrial targeting sequence (MTS).

Progressive External Ophthalmoplegia (PEO)

[0378] PEO is a slowly progressive disorder associated with slow eye movement speed, limited gaze in all directions, ptosis, and extraocular muscle pathology. PEO may arise sporadically or as a consequence of autosomal dominant, autosomal recessive, or maternal inheritance.

1. Sporadic PEO

[0379] Syndromes with severe ophthalmoplegia include Kearns-Sayre, PEO + Proximal myopathy, and PEO.

[0380] Chronic PEO is caused by a single large mtDNA deletion and usually manifests at > 20 years of age. Symptoms include ophthalmoplegia, and heart block in some patients.

[0381] PEO with sensory ataxic neuropathy usually manifests between 10 to 31 years of age. Clinical features include sensory loss, gait disorder, distal motor weakness, absent tendon reflexes, external ophthalmoplegia, ptosis, dysarthria, facial weakness, myopathy, and ragged red muscle fibers.

[0382] Mutations in MTTQ can result in PEO that presents at 5 years of age. Clinical symptoms include weakness, ptosis, dysphonia, dysphagia, ophthalmoplegia, reduced tendon reflexes, ragged red fibers, COX negative muscle fibers and impairment in mitochondrial protein synthesis.

[0383] Mutations in MTTA can result in PEO that presents in the 6th decade of life. Clinical symptoms include ptosis, weakness, decreased eye movements, dysphagia, COX negative muscle fibers, mitochondrial proliferation, and partial defect of Complex I.

[0384] Mutations in MTTL can result in PEO that presents in the 5th decade of life. Clinical symptoms include ptosis, migraines, decreased eye movements, exercise intolerance, short stature, COX negative, ragged red muscle fibers, and partial defects of Complex I & IV.

[0385] Mutations in MTTY can result in PEO that presents in the 4th decade of life. Clinical symptoms include ptosis, exercise intolerance, ophthalmoplegia, myopathy, COX negative muscle fibers with increased SDH staining, and partial defect of Complex I & IV. Other MTTY syndromes include exercise intolerance with Complex III deficiency, and focal segmental glomerulosclerosis and dilated cardiomyopathy.

2. Maternally-inherited PEO

[0386] Maternal PEO is caused by mtDNA point mutations in MTTL, MTTN, MTTQ, MTTA, and MTTK.

3. Autosomal Dominant PEO

[0387] Autosomal dominant progressive external ophthalmoplegia (adPEO) with mitochondrial DNA (mtDNA) deletions-3 (PEOA3) is caused by heterozygous mutations in the nuclear-encoded twinkle gene (C10ORF2), which binds to the β-subunit of polymerase-γ (POLG). Progressive external ophthalmoplegia is characterized by multiple mitochondrial DNA deletions in skeletal muscle. The most common clinical features include adult onset of weakness of the external eye muscles and exercise intolerance. Patients with C10ORF2-linked adPEO may have other clinical features including proximal muscle weakness, muscle pain, cramps, respiratory failure, ataxia, peripheral neuropathy, cardiomyopathy, cataracts, depression, ptosis, dysarthria, dysphagia, dysphonia, hearing loss, memory loss, Parkinsonism, avoidant personality traits, SDH+ COX negative muscle fibers, ragged red fibers, ketoacidosis, cortical atrophy or white matter lesions, and endocrine abnormalities. Variant syndromes involving Twinkle mutations include Infantile Onset Spinocerebellar Ataxia (IOSCA), SANDO, MTDPS7, PEO + Dementia, PEO + Parkinson, and Perrault.

[0388] Autosomal dominant progressive external ophthalmoplegia (adPEO) with mitochondrial DNA (mtDNA) deletions-2 (PEOA2) is caused by heterozygous mutations in the nuclear-encoded ANT1 gene (SLC25A4), which usually manifests at 20 to 35 years of age. Clinical symptoms include ophthalmoplegia, ptosis, dysphagia, dysphonia, face, proximal, and respiratory weakness, cataracts, sensorineural hypoacusia, goiter, dementia,

Bipolar affective disorder, high serum lactic acid, and multiple mtDNA deletions. Over-expression of ANT1 is also observed in FSH dystrophy muscle.

[0389] PEOA2 can also be caused by heterozygous mutations in the nuclear-encoded twinkle gene (C10ORF2). The most common mutation is an Ala359Thr missense mutation, the homozygous version producing more severe effects than the heterozygous version. In addition, adPEO is characterized by multiple mitochondrial DNA deletions in skeletal muscle. A severe CNS phenotype with polyneuropathy is associated with a 39-bp deletion. In general, the mutations tend to cluster in regions of the protein involved in subunit interactions (amino acids 303-508). The twinkle protein is involved in mtDNA metabolism and could function as an adenine nucleotide-dependent DNA helicase. The function of the twinkle protein is believed to be critical for lifetime maintenance of mtDNA integrity. The most common clinical features of adPEO include adult onset of weakness of the external eye muscles and exercise intolerance. Patients with C10ORF2-linked adPEO may have other clinical features including proximal muscle weakness, ataxia, peripheral neuropathy, cardiomyopathy, cataracts, depression, and endocrine abnormalities.

[0390] Autosomal dominant progressive external ophthalmoplegia (adPEO) with mitochondrial DNA (mtDNA) deletions-1 (PEOA1) is caused by mutations in the nuclear-encoded DNA polymerase-gamma gene (POLG). Autosomal recessive PEO (PEOB) is also caused by mutations in the POLG gene. PEO1 manifests at 16 to 39 years of age. Clinical features include PEO, muscle weakness, exercise intolerance, sensory loss, absent tendon reflexes, poorly formed 2° sexual characteristics, early menopause, testicular atrophy, Parkinsonism, proximal weakness & wasting, dysphagia, dysphonia, facial diplegia, abnormal gait, depression, extrapyramidal syndrome, ragged red fibers, COX negative and SDH + fibers, and proximal myopathy. Other clinical syndromes associated with dominant POLG mutations include PEO+ Demyelinating neuropathy, PEO + Distal myopathy, Sensory neuropathy, PEO & Tremor, and PEO + Hypogonadism. Clinical syndromes associated with recessive POLG mutations include Alpers-Huttenlocher Syndrome (AHS), Childhood myocerebrohepatopathy spectrum (MCHS), Myoclonic epilepsy, Myopathy, Sensory ataxia (MEMSA), SANDO, MIRAS, MNGIE and Parkinsons.

[0391] PEO+ Demyelinating neuropathy manifests at the second decade of life and is characterized by weakness, sensory loss, absent tendon reflexes, PEO with ptosis, dysphonia,

dysphagia, nerve pathology, ragged red fibers, COX negative fibers, and reduced Complex I, III & IV activity.

[0392] Mitochondrial Recessive Ataxia Syndrome (MIRAS) usually manifests between the ages of 5 to 38 years. Clinical symptoms include balance disorder, epilepsy, dysarthria, nystagmus, reduced tendon reflexes, pain, sensory neuropathy, cramps, cognitive impairment, athetosis, tremor, obesity, and eye movement disorders.

[0393] PEO + Hypogonadism is characterized by delayed sexual maturation, primary amenorrhea, early menopause, testicular atrophy, cataracts, cerebellar ataxia, tremor, Parkinsonism, depression, mental retardation, polyneuropathy, PEO, dysarthria, dysphonia, proximal weakness, rhabdomyolysis, hypoacusis, Pes cavus, ragged red fibers, and cytochrome c oxidase negative muscle fibers.

[0394] Distal myopathy, Cachexia & PEO is caused by dominant or sporadic mutations in POLG1 and usually manifests between the third and fourth decade of life. Clinical features include weakness, dysarthria, dysphagia, cachexia, ptosis, ophthalmoplegia, cataracts, and ragged red & COX negative muscle fibers.

[0395] Autosomal dominant progressive external ophthalmoplegia (adPEO) with mitochondrial DNA (mtDNA) deletions-4 (PEOA4) is caused by heterozygous mutations in the nuclear-encoded DNA polymerase gamma-2 gene (POLG2). Progressive external ophthalmoplegia-4 is an autosomal dominant form of mitochondrial disease that variably affects skeletal muscle, the nervous system, the liver, and the gastrointestinal tract. Age of onset ranges from infancy to adulthood. The phenotype ranges from relatively mild, with adult-onset skeletal muscle weakness and weakness of the external eye muscles, to severe, with a multisystem disorder characterized by delayed psychomotor development, lactic acidosis, constipation, and liver involvement. Clinical features include ptosis, external ophthalmoplegia, exercise intolerance, pain, weakness, seizures, hypotonia, impaired glucose tolerance, high lactate, cerebellar atrophy, cardiac conduction defect, and abnormal mitochondrial morphology.

[0396] Autosomal dominant progressive external ophthalmoplegia-6 (PEOA6) is caused by heterozygous mutations in the DNA2 gene. PEOA6 is characterized by muscle weakness, mainly affecting the lower limbs, external ophthalmoplegia, exercise intolerance, and mitochondrial DNA (mtDNA) deletions on muscle biopsy. Clinical features include

hypotonia, myalgia, exertional dyspnea, ptosis or ophthalmoplegia, lordosis, and muscular atrophy. Symptoms may appear in childhood or adulthood and show slow progression.

[0397] In some embodiments, dominant POLG mutations may lead to sensory neuropathy, tremor and PEO.

4. Autosomal Recessive PEO

[0398] PEO + Myopathy & Parkinsonism is an adult onset autosomal recessive disorder. Clinical features include extrapyramidal signs (*e.g.*, akinesia, rigidity, rest tremor), ptosis, ophthalmoplegia, proximal & facial weakness, occasional distal leg weakness, hearing loss, SDH + and COX negative muscle fibers, reduced complex III activity, and multiple mtDNA deletions.

[0399] Autosomal recessive progressive external ophthalmoplegia (PEOB) is caused by homozygous or compound heterozygous mutations in the nuclear-encoded DNA polymerase-gamma gene (POLG). Recessive mutations in the POLG gene can also cause sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO), which shows overlapping features. Autosomal recessive PEO is usually more severe than autosomal dominant PEO.

[0400] SANDO usually manifests between the ages of 16 to 38 years and is characterized by exercise intolerance, ptosis, and paresthesias. Clinical symptoms include sensory loss, ataxic gait, pseudoathetosis, small fiber modality loss, weakness, reduced tendon reflexes, ptosis, ophthalmoplegia, dysarthria, myoclonic epilepsy, depression, high CSF and serum lactate, degeneration of spinocerebellar and dorsal column tracts, thalamic lesions, cerebellar atrophy or White matter lesions, ragged red fibers, loss of myelinated & unmyelinated axons, and reduced activity of Complex I & IV.

[0401] Mitochondrial DNA Depletion Syndrome-11 (MTDPS11) can be caused by homozygous mutations in the MGME1 gene. Mitochondrial DNA Depletion Syndrome-11 is an autosomal recessive mitochondrial disorder characterized by onset in childhood or adulthood of progressive external ophthalmoplegia (PEO), ptosis, muscle weakness and atrophy, exercise intolerance, dysphonia, dysphagia, and respiratory insufficiency due to muscle weakness. More variable features include spinal deformity, emaciation, and cardiac abnormalities. Skeletal muscle biopsies show deletion and depletion of mitochondrial DNA (mtDNA) with variable defects in respiratory chain enzyme activities. Additional features

include scapular winging, mental retardation, memory deficits, nausea, flatulence, abdominal fullness, diarrhea, loss of appetite, SDH+ & COX negative fibers, Complex I or I + IV deficiencies, and cerebellar atrophy.

[0402] PEO with cardiomyopathy is caused by recessive mutations in POLG and usually manifests at childhood. Clinical features include PEO, cardiomyopathy, proximal weakness, multiple mtDNA deletions, and ragged red fibers.

[0403] An additional POLG syndrome is Parkinsonism without external ophthalmoplegia. Clinical feature include Parkinsonism or Dystonia, weakness, high serum lactate, COX negative muscle fibers, polyneuropathy, and cerebral and cerebellar atrophy.

[0404] Ataxia with sensory neuropathy is a variant POLG syndrome that is not associated with ophthalmoplegia.

PEPCK Deficiency

[0405] PEPCK deficiency is an autosomal recessive disorder of carbohydrate metabolism. A deficiency of the enzyme phosphoenolpyruvate carboxykinase (PEPCK), which is a key enzyme in gluconeogenesis, causes acidemia. PEPCK converts oxaloacetate into phosphoenolpyruvate and carbon dioxide. PEPCK deficiency is characterized by hypoglycemia, hypotonia, hepatomegaly, liver impairment, and failure to thrive. In humans, there are two forms of PEPCK deficiency: cytosolic and mitochondrial. Both forms result from an inherited deficiency in the enzyme PEPCK. Cytosolic PEPCK is encoded by PCK1 and the mitochondrial enzyme is encoded by PCK2. PCK2 encodes a deduced 640-amino acid polypeptide that shares 70% homology with cytosolic PCK.

Perrault Syndromes

[0406] Perrault Syndrome (PRLTS) is a sex-influenced, autosomal recessive disorder characterized by sensorineural deafness in both males and females and premature ovarian failure (POF) secondary to ovarian dysgenesis in females. Some patients also have neurologic manifestations, including mild mental retardation and cerebellar and peripheral nervous system involvement. Perrault Syndrome is classified into type I, which is static and without neurologic disease, and type II, which is with progressive neurologic disease.

[0407] Perrault Syndrome-1 (PRLTS1) is caused by compound heterozygous mutations in the HSD17B4 gene, which encodes a D-bifunctional protein (DBP).

[0408] Perrault Syndrome-2 (PRLTS2) is caused by compound heterozygous mutations in the mitochondrial histidyl-tRNA synthetase, HARS2. Affected females have primary amenorrhea, streak gonads, and infertility, whereas affected males show normal pubertal development and are fertile

[0409] Perrault Syndrome-3 (PRLTS3) is caused by homozygous or compound heterozygous mutations in the CLPP gene, an endopeptidase component of a mitochondrial ATP-dependent proteolytic complex required for protein degradation in the mitochondria.

[0410] Perrault Syndrome-4 (PRLTS4) is caused by homozygous or compound heterozygous mutations in the LARS2 gene.

[0411] Mutations in the Twinkle gene can also lead to Perrault Syndrome. In addition to sensorineural deafness and female hypogonadism, patients exhibit symptoms such as nystagmus, gait disorder, epilepsy, polyneuropathy, ophthalmoplegia, increased serum lactate, and muscle atrophy.

Propionic Acidemia

[0412] Propionic acidemia (PA) is caused by a deficiency of propionyl-CoA carboxylase (PCC), a biotin-dependent carboxylase located in the mitochondrial inner membrane space. PCC catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA, which eventually enters the TCA cycle as succinyl-CoA. Propionyl-CoA is common to the pathway for degradation of some amino acids (isoleucine, valine, threonine, and methionine), odd-chain fatty acids, and cholesterol. Gut bacteria (*i.e.*, Propionibacterium sp.) are also an important source of propionate metabolized through PCC. PCC is a heterododecamer (α6β6) composed of six α-subunits encoded by PCCA and six β-subunits encoded by PCCB. Biallelic mutation of either PCCA or PCCB results in PA.

[0413] Patients with PA exhibit episodic vomiting, lethargy, ketosis, neutropenia, periodic thrombocytopenia, hypogammaglobulinemia, developmental retardation, and intolerance to protein. Chemical features include hyperglycinemia and hyperglycinuria.

Pyruvate Disorders

[0414] Pyruvate dehydrogenase complex (PDHC) is a nuclear-encoded mitochondrial matrix multienzyme complex composed of multiple copies of 3 enzymes: E1, Dihydrolipoyl transacetylase (DLAT), and Dihydrolipoyl dehydrogenase (DLD). PDHC catalyzes the

irreversible conversion of pyruvate into acetyl-CoA. Pyruvate disorders may arise as a consequence of perturbations in PDHA1, PDHB, PDHX, PDK3, PDP1, DLAT, DLD, NFU1, BOLA3, Lipoic acid synthase (LIAS), TPK1, Pyruvate carboxylase and Pyruvate transporter.

1. Pyruvate Carboxylase Deficiency

[0415] Pyruvate carboxylase converts pyruvate & CO_2 to oxaloacetate and plays a role in gluconeogenesis. Pyruvate carboxylase deficiency is caused by mutations in the pyruvate carboxylase gene and is categorized into 3 phenotypic subgroups: Type A, Type B and Type C. Type A patients have lactic acidemia and psychomotor retardation, whereas Type B patients have a more complex biochemical phenotype with increased serum lactate, ammonia, citrulline, and lysine, as well as an intracellular redox disturbance in which the cytosolic compartment is more reduced and the mitochondrial compartment is more oxidized. Type B patients have decreased survival compared to group A, and usually do not survive beyond 3 months of age. Type C is relatively benign. Clinical features include hypotonia, delayed neurologic development, ataxia, chronic lactic acidemia, developmental delay, seizures, lactic acidosis, increased lactate:pyruvate & acetoacetate:3-hydroxybutyrate ratios, and episodic metabolic acidosis.

2. Pyruvate Dehydrogenase E1-α Deficiency (PDHAD)

[0416] Pyruvate dehydrogenase E1-alpha deficiency (PDHAD) is caused by mutations in the gene encoding the E1-alpha polypeptide (PDHA1) of the pyruvate dehydrogenase (PDH) complex. Genetic defects in the pyruvate dehydrogenase complex are one of the most common causes of primary lactic acidosis in children. Most cases are caused by mutation in the E1-alpha subunit gene on the X chromosome. X-linked PDH deficiency is one of the few X-linked diseases in which a high proportion of heterozygous females manifest severe symptoms. Clinical features of PDHAD include seizures, hyperventilation, episodic cerebellar ataxia, chorioathetosis, lactic acidosis, carbohydrate intolerance, high serum pyruvic acid, and high serum alanine.

[0417] Variant syndromes associated with Pyruvate dehydrogenase E1-alpha deficiency are also common and are characterized by hypotonia, lethargy, seizures, dystonia, psychomotor retardation, Leigh-like lesions and hyperlactataemia.

3. Pyruvate Dehydrogenase E1-β Deficiency (PDHBD)

[0418] Pyruvate dehydrogenase E1-beta deficiency (PDHBD) is caused by homozygous mutations in the PDHB gene, and typically presents at the infant stage. Clinical symptoms include hypotonia, respiratory insufficiency, lactic acidosis, corpus callosum agenesis, and reduced PDH activity.

4. Dihydrolipoamide Dehydrogenase (DLD) Deficiency

[0419] The DLD gene encodes dihydrolipoamide dehydrogenase (EC 1.8.1.4), a flavoprotein component known as E3 that is common to the 3 alpha-ketoacid dehydrogenase multienzyme complexes, namely, pyruvate dehydrogenase complex, the alpha-ketoglutarate dehydrogenase complex (KGDC), and the branched-chain alpha-keto acid dehydrogenase complex (BCKDC). The enzyme is a functional homodimer of the DLD protein and catalyzes the oxidative regeneration of a lipoic acid cofactor covalently bound to E2 (DBT) yielding NADH. The DLD enzyme is also a component, referred to as the L protein, of the mitochondrial glycine cleavage system (GCS). Clinical symptoms include vomiting and abdominal pain, stroke-like episodes, hypothermia, motor retardation, myoglobinuria, exertional fatigue, lactic acidosis, hypoglycemia, and high pyruvate, lactate, α-ketoglutarate, and branched-chain amino acids.

5. Pyruvate Dehydrogenase Phosphatase Deficiency (PDHPD)

[0420] Pyruvate dehydrogenase phosphatase deficiency can be caused by mutations in the PDP1 gene. Clinical features include hypotonia, developmental delay, seizures, lactic acidosis, and posterior white matter pathology.

6. Pyruvate Dehydrogenase E3-binding Protein Deficiency (PDHXD)

[0421] Pyruvate dehydrogenase E3-binding protein deficiency is caused by homozygous or compound heterozygous mutations in the PDHX gene. Clinical features include hypotonia, psychomotor retardation, Leigh Syndrome, optic atrophy, diplegia, dysarthria, lactic acidosis, putaminal lesions, and hemolytic anemia.

7. Mitochondrial Pyruvate Carrier Deficiency (MPYCD)

[0422] Mitochondrial pyruvate carrier deficiency (MPYCD) is an autosomal recessive metabolic disorder characterized by delayed psychomotor development and lactic acidosis with a normal lactate/pyruvate ratio resulting from impaired mitochondrial pyruvate

oxidation. MPYCD is caused by homozygous mutations in the BRP44L gene and usually manifests at birth or childhood. Clinical features include hypotonia, psychomotor retardation, peripheral neuropathy, dysmorphic features (face, single palmar fold, wide spaced nipples), hepatomegaly, metabolic acidosis, hyperlactacidemia, and periventricular cysts.

Schwartz-Jampel Syndrome Type 1 (SJS1)

[0423] Schwartz-Jampel Syndrome type 1 (SJS1) is caused by mutations in the gene encoding perlecan (HSPG2), a heparan sulfate proteoglycan. Perlecan is a major component of basement membranes & interstitial matrix in cartilage and functions as a coreceptor for FGF2.

[0424] Schwartz-Jampel Syndrome type 1A occurs during childhood (usually < 3 years). Clinical features include respiratory difficulties, impaired swallowing, polyhydramnios, absent stomach bubble, short femurs, skeletal contractures, muscle stiffness, reduced tendon reflexes, muscle hypertrophy, malignant hyperthermia, mental retardation, bone dysplasia, micrognathia, platyspondyly, cleft vertebrae, reduced height, kyphoscoliosis, myopia, cataracts, blepharophimosis, medial displacement of outer canthi, hirsutism, small testes, microstomia, jaw muscle rigidity. SJS Type 1B occurs at birth and is associated with more severe bone dysplasia. Silverman-Handmaker type of dyssegmental dysplasia (DDSH) refers to an allelic disorder with a more severe phenotype.

Selenium Deficiency

[0425] Selenium deficiency results in reduced glutathione peroxidase activity, oxidative damage, reduced levels of selenoproteins and increased toxicity of drugs & toxins (*e.g.*, nitrofurantoin, paraquat). Clinical features include myopathy, cardiomyopathy, selenoprotein disorders (congenital muscular dystrophy with rigid spine, hyperthyroxinemia), nail and hair loss, gastroenteritis, dermatitis, malabsorption, muscle pain, high serum creatine kinase, low vitamin E levels, and enlarged mitochondria.

Short-chain Acyl-CoA Dehydrogenase deficiency

[0426] Short-chain acyl-CoA dehydrogenase (SCAD) deficiency is an autosomal recessive metabolic disorder of mitochondrial fatty acid beta-oxidation. The disorder is associated with mutations in the ACADS gene encoding short-chain acyl-CoA dehydrogenase. Two clinical phenotypes of SCAD deficiency have been identified. One form of the disorder is an

infantile onset characterized by acute acidosis, myopathy, failure to thrive, developmental delay, and seizures. The other form is observed in middle-aged patients who exhibit chronic myopathy, ophthalmoplegia, ptosis, and scoliosis.

Succinyl CoA:3-oxacid CoA Transferase Deficiency

[0427] Succinyl CoA:3-oxacid CoA transferase (SCOT) deficiency is an inborn error of ketone body metabolism associated with mutations in the OXCT1 gene. SCOT is a key mitochondrial enzyme in the metabolism of ketone bodies in various organs. Deficiency of SCOT activity inhibits peripheral ketone body utilization and causes episodes of severe ketoacidosis. Ketones are molecules produced in the liver during the breakdown of fats and are the major vectors of energy transfer from the liver to extrahepatic tissues. As the first step of ketone body utilization, SCOT catalyzes the reversible transfer of CoA from succinyl-CoA to acetoacetate.

Stuve-Wiedemann Syndrome (STWS)

[0428] Stuve-Wiedemann Syndrome (STWS), also known as neonatal Schwartz-Jampel Syndrome type 2 (SJS2), is caused by a mutation in the leukemia inhibitory factor receptor gene (LIFR).

[0429] Stuve-Wiedemann Syndrome (STWS) is an autosomal recessive disorder characterized by bowing of the long bones and other skeletal anomalies, episodic hyperthermia, and respiratory and feeding distress usually resulting in early death. Age of onset is typically at birth. Clinical features include hypotonia, respiratory & feeding difficulties, hyperthermic episodes, high mortality in infancy, joint contractures, bent bone dysplasia, bowing of lower limbs, internal cortical thickening, wide metaphyses, camptodactyly, spontaneous fractures, short stature, malignant hyperthermia, temperature instability, loss of corneal reflex, smooth tongue, reduced tendon reflexes, and reduced Complex I and IV activity.

Thrombocytopenia

[0430] Thrombocytopenia (THC) is characterized by a decrease in platelet count, resulting in the potential for increased bleeding and a decreased clotting ability. Although inherited forms of this syndrome are relatively rare, a number of genes underlying thrombocytopenia have been identified. One form of autosomal dominant nonsyndromic thrombocytopenia is

caused by a mutation in the CYCS gene encoding cytochrome c. Cytochrome c is located in the mitochondria of all aerobic cells and is involved in the electron transport chain that functions in oxidative phosphorylation. Mutations in the CYCS gene have been shown to increase the apoptotic activity of cytochrome c in individuals with autosomal dominant nonsyndromic thrombocytopenia.

Very Long-chain Acyl-CoA Dehydrogenase Deficiency

[0431] Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is a disorder of fatty acid oxidation associated with the accumulation of fatty acids and decreases in cell energy metabolism due to enzyme defects in the fatty acid metabolic pathway. VLCADD is caused by homozygous or compound heterozygous mutations in the ACADVL gene that encodes very long-chain acyl-CoA dehydrogenase. Very long-chain acyl-CoA dehydrogenase (VLCAD) is unique among the acyl-CoA dehydrogenases in its size, structure, and intramitochondrial distribution. Whereas other acyl-CoA dehydrogenases are homotetramers of a 43- to 45-kD subunit, VLCAD has been shown to be a 154-kD homodimer of a 70-kD subunit. VLCAD has been found to be loosely bound to the mitochondrial inner membrane and required detergent for stabilization. By contrast, the other three acyl-CoA dehydrogenases are readily extractable without detergent, indicating that they are located in the mitochondrial matrix.

[0432] VLCAD deficiency is classified into three forms: a severe early-onset with a high incidence of cardiomyopathy and high mortality; an intermediate form with childhood onset, usually with hypoketotic hypoglycemia and a more favorable outcome; and an adult-onset, myopathic form with isolated skeletal muscle involvement, rhabdomyolysis, and myoglobinuria after exercise or fasting.

Vitamin D-dependent Rickets Type 1A

[0433] Vitamin D-dependent rickets type 1A (VDDR1A) is an autosomal recessive disorder characterized by hypocalcemia, secondary hyperparathyroidism, and early onset severe rickets. The disorder is caused by a mutation in the CYP27B1 gene encoding the enzyme 25-hydroxyvitamin D3-1-alpha-hydroxylase, which is localized to the mitochondrial inner membrane. 25-hydroxyvitamin D3-1-alpha-hydroxylase is expressed in the renal proximal tubule where it catalyzes the hydroxylation of 25-hydroxyvitamin D3 into 1-alpha,25-dihydroxyvitamin D3 (1,25$(OH)_2$D3, or calcitrol). The active metabolite 1,25$(OH)_2$D3 binds

and activates the nuclear vitamin D receptor (VDR) and regulates physiologic events such as calcium homeostasis and cellular differentiation and proliferation.

Wilson's Disease

[0434] Wilson's disease is caused by homozygous or compound heterozygous mutations in the ATP7B gene. Wilson's disease is an autosomal recessive disorder characterized by dramatic build-up of intracellular hepatic copper with subsequent hepatic and neurologic abnormalities. In Wilson disease, the basal ganglia and liver undergo changes that express themselves in neurologic manifestations and signs of cirrhosis, respectively. Markedly reduced levels of cytochrome oxidase activity and low ceruloplasmin serum levels are observed in affected individuals. Ceruloplasmin functions in enzymatic transfer of copper to copper-containing enzymes such as cytochrome oxidase.

[0435] There are at least 3 forms of Wilson disease. In a rare 'atypical form,' the heterozygotes show about 50% of the normal level of ceruloplasmin. In the 2 typical forms, the Slavic and the juvenile type, heterozygotes have normal ceruloplasmin levels, although they can be identified by decreased reappearance of radioactive copper into serum and ceruloplasmin. The Slavic type has a late age of onset and is predominantly a neurologic disease. The juvenile type, which occurs in Western Europeans and several other ethnic groups, has onset before age 16 years and is frequently a hepatic disease.

[0436] The Kayser-Fleischer ring is a deep copper-colored ring at the periphery of the cornea which is frequently found in Wilson disease and is thought to represent copper deposits. Additional clinical symptoms include azure lunulae of the fingernails, hypercalciuria, nephrocalcinosis, renal stones, nephrolithiasis, chondrocalcinosis, osteoarthritis, hemolytic anemia, leukoencephalopathy, neuropathy, respiratory chain defects, myocardial abnormalities, intermittent paresthesia and weakness in both hands and feet.

Peroxisome Biogenesis Disorder 3A (PBD3A): Zellweger Syndrome

[0437] Zellweger Syndrome (PBD3A) is caused by homozygous or compound heterozygous mutations in the PEX12 gene on chromosome 17. The peroxisomal biogenesis disorder (PBD) Zellweger Syndrome (ZS) is an autosomal recessive multiple congenital anomaly syndrome resulting from disordered peroxisome biogenesis. Affected children present in the newborn period with profound hypotonia, seizures, and inability to feed. Characteristic craniofacial anomalies, eye abnormalities, neuronal migration defects,

hepatomegaly, and chondrodysplasia punctata are present. Children with this condition do not show any significant development and usually die in the first year of life. Brain MRIs of affected subjects show reduced white matter and hypoplasia in corpus callosum.

[0438] Another form of peroxisome biogenesis disorder (PBD8B) is caused by homozygous mutations in the PEX16 gene. Mutations in PEX16 also cause Zellweger Syndrome. The age of onset usually occurs between 1 to 2 years. Clinical symptoms are progressive and include spasticity, dysarthria, dysphagia, ataxia, abnormal gait, delayed walking, optic atrophy, cataracts, constipation, and neuropathy.

[0439] The overlapping phenotypes of neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD) represent the milder manifestations of the Zellweger Syndrome spectrum (ZSS) of peroxisome biogenesis disorders. The clinical course of patients with the NALD and IRD presentation is variable and may include developmental delay, hypotonia, liver dysfunction, sensorineural hearing loss, retinal dystrophy, and visual impairment. Children with the NALD presentation may reach their teens, and those with the IRD presentation may reach adulthood.

Mitochondrial Disorders Associated with Drugs and Toxins

1. Arsenic Trioxide Myopathy

[0440] Arsenic trioxide (ATO) has a proven therapeutic efficacy in acute promyelocytic leukemia (APL). However, APL patient who have undergone ATO treatment may develop a delayed, severe, and partially reversible mitochondrial myopathy. Affected individuals may present with the inability to walk, myopathy with cytoplasmic lipid droplets, decreased mitochondrial respiratory chain complex activity, multiple mtDNA deletions, and elevated muscle arsenic content.

2. Myopathy and Neuropathy Resulting from Nucleoside Analogues

[0441] Nucleoside analogues are molecules that function as nucleosides in DNA or RNA replication. These analogs include a range of antiviral products used in the prevention of viral replication. Various nucleoside analogues including azidothymidine (AZT), clevudine, telbivudine, and fialuridine are associated with the development myopathies and neuropathies.

3. Germanium Myopathy

[0442] Germanium can have a toxic effect on skeletal muscle leading to myopathy and polyneuropathy. Pathological examinations of skeletal muscle from individuals affected by germanium intoxication exhibit vacuolar myopathy with lipid excess, increased acid phosphatase activity, decreased cytochrome oxidase activity, and mitochondrial abnormalities.

4. Parkinsonism and Mitochondrial Complex I Neurotoxicity due to Trichloroethylene

[0443] Long-term exposure to trichloroethylene is associated with Parkinsonism and mitochondrial Complex I neurotoxicity. Neurotoxic actions of trichloroethylene include selective Complex 1 impairment in the midbrain with concomitant striatonigral fiber degeneration and loss of dopamine neurons.

5. Valproate-induced Hepatic Failure

[0444] Valproate is an anticonvulsant that is administered to control certain types of seizures in the treatment of epilepsy. There is an increased risk of Valproate-induced liver failure in patients with hereditary neurometabolic syndromes caused by mutations of the mitochondrial DNA polymerase γ (POLG) gene.

Other Mitochondrial Syndromes Characterized by Infantile and Childhood Onset

1. Neurodegeneration with Brain Iron Accumulation

[0445] Neurodegeneration with brain iron accumulation-4 (NBIA4) is an autosomal recessive neurodegenerative disorder caused by a homozygous or compound heterozygous mutation in the C19ORF12 gene. Neurodegeneration with brain iron accumulation-1 (NBIA1), also known as Hallervorden-Spatz disease, is caused by a homozygous or compound heterozygous mutation in the pantothenate kinase-2 gene, PANK2.

2. Primary Coenzyme Q10 Deficiency-3

[0446] Primary coenzyme Q10 deficiency-3 (COQ10D3) is a fatal encephalomyopathic form of coenzyme Q10 deficiency with nephrotic syndrome that can be caused by compound heterozygous mutation in the PDSS2 gene, which encodes a subunit of decaprenyl diphosphate synthase, the first enzyme of the CoQ10 biosynthetic pathway.

3. Combined Mitochondrial Complex Deficiencies

[0447] Combined complex deficiencies include combined complex I, II, IV, ± V deficiency, combined complex I, II, and III deficiency, combined oxidative phosphorylation deficiency-1 (COXPD1), combined oxidative phosphorylation deficiency-2 (COXPD2), combined oxidative phosphorylation deficiency-3 (COXPD3), combined oxidative phosphorylation deficiency-4 (COXPD4), combined oxidative phosphorylation deficiency-5 (COXPD5), combined oxidative phosphorylation deficiency-6 (COXPD6), combined oxidative phosphorylation deficiency-7 (COXPD7), combined oxidative phosphorylation deficiency-8 (COXPD8), combined oxidative phosphorylation deficiency-9 (COXPD9), combined oxidative phosphorylation deficiency-10 (COXPD10), combined oxidative phosphorylation deficiency-11 (COXPD11), combined oxidative phosphorylation deficiency-12 (COXPD12), combined oxidative phosphorylation deficiency-13 (COXPD13), combined oxidative phosphorylation deficiency-14 (COXPD14), combined oxidative phosphorylation deficiency-15 (COXPD15), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-17 (COXPD17), combined oxidative phosphorylation deficiency-18 (COXPD18), combined oxidative phosphorylation deficiency-19 (COXPD19), combined oxidative phosphorylation deficiency-20 (COXPD20), combined oxidative phosphorylation deficiency-21 (COXPD21), mitochondrial DNA depletion myopathy (MTDPS2), and Multiple Mitochondrial Dysfunctions Syndrome (MMDS).

4. Complex I Deficiency

[0448] Mitochondrial Complex I (NADH-ubiquinone reductase) deficiency is associated with several disorders including cardiomyopathy due to mutations in the NDUFS2 gene, fatal multisystemic complex I deficiency due to mutations in the NDUFS4 gene, and lethal infantile mitochondrial disease due to mutations in the NDUFS6, C20ORF7, NDUFAF3, and/or NDUFB2 genes.

5. Complex II Deficiency

[0449] Mitochondrial Complex II (succinate dehydrogenase-CoQ oxoreductase) deficiency is associated with disorders including Leigh Syndrome due to mutations in the SDHA gene, infantile leukoencephalopathy due to SDHAF1 mutations, iron-sulfur disorders, paragangliomas and pheochromocytomas due to mutations in the SDHAF2, SDHB, SDHC, and SDHD genes.

6. Complex III Deficiency

[0450] Mitochondrial Complex III (cytochrome reductase) deficiency is associated with disorders including insulin-responsive hyperglycemia and encephalopathy due to mutations in the CYC1 gene and hypoglycemia due to mutations in the UQCRB gene.

7. Complex IV Deficiency

[0451] Complex IV (cytochrome oxidase) deficiency is associated with disorders including developmental delay and multisystem disorders due to mutations in CEP89, hepatic failure due to mutations in SCO1, leukodystrophies due to mutations in COX6B1 and/or APOPT1, and spastic ataxia due to mutations in COX10.

8. Complex V Deficiency

[0452] Complex V (ATP synthase) deficiency is associated with disorders including apical hypertrophic cardiomyopathy due to mutations in MT-ATP8, dysmorphic cerebrooculofacioskeletal features due to mutations in ATPAF2, and mental retardation, polyneuropathy, and episodic lactic acidosis due to mutations in TTP5E.

9. Fumarase Deficiency

[0453] Fumarase deficiency, also known as fumaric aciduria, is caused by a homozygous or compound heterozygous mutation in the fumarate hydratase gene (FH). Fumarase deficiency is a severe autosomal recessive metabolic disorder characterized by early-onset hypotonia, profound psychomotor retardation, and brain abnormalities, such as agenesis of the corpus callosum, gyral defects, and ventriculomegaly. Many patients show neonatal distress, metabolic acidosis, and/or encephalopathy.

10. 3-Hydroxy-3-methylglutaryl-CoA Synthase-2 Deficiency

[0454] Mitochondrial HMG-CoA synthase-2 deficiency is caused by mutation in the gene encoding mitochondrial HMG-CoA synthase-2 (HMGCS2). Mitochondrial HMG-CoA synthase deficiency is an inherited metabolic disorder caused by a defect in the enzyme that regulates the formation of ketone bodies. Patients present with hypoketotic hypoglycemia, encephalopathy, and hepatomegaly, usually precipitated by an intercurrent infection or prolonged fasting.

11. Hyperuricemia, Pulmonary Hypertension, Renal Failure, and Alkalosis Syndrome

[0455] Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (HUPRA) Syndrome is caused by homozygous mutation in the SARS2 gene, which encodes mitochondrial seryl-tRNA synthetase. HUPRA Syndrome is a severe autosomal recessive multisystem disorder characterized by onset in infancy of progressive renal failure leading to electrolyte imbalances, metabolic alkalosis, pulmonary hypertension, hypotonia, and delayed development. Affected individuals are born prematurely.

12. Linear Skin Lesions

[0456] Linear skin lesions are associated with syndromic microphthalmia-7 (MCOPS7) and reticulolinear aplasia cutis congenita with microcephaly, facial dysmorphism, and other congenital anomalies (APLCC).

[0457] Syndromic microphthalmia-7 is caused by mutation in the HCCS gene. The microphthalmia with linear skin defects syndrome (MLS) is an X-linked dominant disorder characterized by unilateral or bilateral microphthalmia and linear skin defects—which are limited to the face and neck, consisting of areas of aplastic skin that heal with age to form hyperpigmented areas—in affected females and in utero lethality for males. A similar form of congenital linear skin defects, also limited to the face and neck and associated with microcephaly, is APLCC, which can be caused by mutation in the COX7B gene.

13. Pontocerebellar Hypoplasia Type 6

[0458] Pontocerebellar hypoplasia (PCH) is a heterogeneous group of disorders characterized by an abnormally small cerebellum and brainstem and associated with severe developmental delay. Pontocerebellar hypoplasia type 6 (PCH6) is caused by a homozygous or compound heterozygous mutation in the gene encoding mitochondrial arginyl-tRNA synthetase (RARS2).

14. Pyruvate Dehydrogenase Complex Disorders

[0459] Pyruvate dehydrogenase complex (PDHC) disorders are a common cause of lactic acidosis and encephalopathy in children. PDHC disorders are associated with mutations in the following genes: PDHA1, PDHB, PDHX, PDP1, DLD, DLAT, LIAS, and TPK1.

15. Mitochondrial DNA Depletion Syndrome-9

[0460] Mitochondrial DNA Depletion Syndrome-9 (MTDPS9P), also known as severe neonatal lactic acidosis with mtDNA depletion, is caused by a homozygous or compound heterozygous mutation in the alpha subunit of the succinate-CoA ligase gene (SUCLG1). MTDPS9P is a severe autosomal recessive disorder characterized by infantile onset of hypotonia, lactic acidosis, severe psychomotor retardation, progressive neurologic deterioration, and excretion of methylmalonic acid. Some patients with MTDPS9 die in early infancy.

16. Sudden Infant Death Syndrome

[0461] Sudden infant death Syndrome (SIDS) is associated with mutations identified in MTTL1, which encodes mitochondrial leucine transfer RNA 1, MTND2, which encodes NADH dehydrogenase subunit 1, and HADHB, which encodes the beta subunit of the mitochondrial trifunctional protein.

[0462] In one aspect, the present disclosure provides a method of treating, ameliorating or preventing a mitochondrial disease or disorder or signs and symptoms thereof, comprising administering a therapeutically effective amount of a composition comprising mitochondrial-targeted creatine compounds, or derivatives, analogues, or pharmaceutically acceptable salts thereof. In some embodiments of the method, the mitochondrial disease or disorder is selected from the group consisting of Alexander disease, Alpers Syndrome, Alpha-ketoglutarate dehydrogenase (AKDGH) deficiency, ALS-FTD, Sideroblastic anemia with spinocerebellar ataxia, Pyridoxine-refractory sideroblastic anemia, GRACILE Syndrome, Björnstad Syndrome, Leigh Syndrome, mitochondrial complex III deficiency nuclear type 1 (MC3DN1), combined oxidative phosphorylation deficiency 18 (COXPD18), Thiamine-responsive megaloblastic anemia syndrome (TRMA), Pearson Syndrome, HAM Syndrome, Ataxia, Cataract, and Diabetes Syndrome, MELAS/MERRF Overlap Syndrome, combined oxidative phosphorylation deficiency-14 (COXPD14), Infantile cerebellar-retinal degeneration (ICRD), Charlevoix-Saguenay spastic ataxia, Primary coenzyme Q10 deficiency-1 (COQ10D1), ataxia oculomotor apraxia type 1 (AOA1), Autosomal recessive spinocerebellar ataxia-9/ coenzyme Q10 deficiency-4 (COQ10D4), Ataxia, Pyramidal Syndrome, and Cytochrome Oxidase Deficiency, Friedreich's ataxia, Infantile onset spinocerebellar ataxia (IOSCA)/ Mitochondrial DNA Depletion Syndrome-7, Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation

(LBSL), Autosomal recessive spastic ataxia-3 (SPAX3), MIRAS, SANDO, mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), spastic ataxia with optic atrophy (SPAX4), progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5), mitochondrial complex III deficiency nuclear type 2 (MC3DN2), episodic encephalopathy due to thiamine pyrophosphokinase deficiency/Thiamine Metabolism Dysfunction Syndrome-5 (THMD5), Spinocerebellar ataxia-28 (SCA28), autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN), Dominant Optic Atrophy (DOA), cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) Syndrome, spinocerebellar ataxia 7 (SCA7), Barth Syndrome, Biotinidase deficiency, gyrate atrophy, Syndromic Dominant Optic Atrophy and Deafness (Syndromic DOAD), Dominant Optic Atrophy plus (DOAplus), Leber's hereditary optic neuropathy (LHON), Wolfram Syndrome-1 (WFS1), Wolfram Syndrome-2 (WFS2), Age-related macular degeneration (ARMD), Brunner Syndrome, Left ventricular noncompaction-1 (LVNC1), histiocytoid cardiomyopathy, Familial Myalgia Syndrome, Parkinsonism, Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency-1 (CEMCOX1), Sengers Syndrome, Cardiofaciocutaneous Syndrome-1 (CFC1), Mitochondrial trifunctional protein (MTP) deficiency, infantile encephalocardiomyopathy with cytochrome c oxidase deficiency, cardiomyopathy + encephalomyopathy, mitochondrial phosphate carrier deficiency, infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency (CEMCOX2), β-Hydroxyisobutyryl CoA Deacylase (HIBCH) deficiency, ECHS1) deficiency, Maternal Inheritance Leigh Syndrome (MILS), dilated cardiomyopathy with ataxia (DCMA), Mitochondrial DNA Depletion Syndrome-12 (MTDPS12), cardiomyopathy due to mitochondrial tRNA deficiencies, mitochondrial complex V (ATP synthase) deficiency nuclear type 1 (MC5DN1), combined oxidative phosphorylation deficiency-8 (COXPD8), progressive leukoencephalopathy with ovarian failure (LKENP), combined oxidative phosphorylation deficiency-10 (COXPD10), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-17 (COXPD17), combined oxidative phosphorylation deficiency-5 (COXPD5), combined oxidative phosphorylation deficiency-9 (COXPD9), carnitine acetyltransferase (CRAT) deficiency, carnitine palmitoyltransferase I (CPT I) deficiency, myopathic carnitine deficiency, primary systemic carnitine deficiency (CDSP), carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acylcarnitine translocase deficiency (CACTD), cartilage-hair hypoplasia, cerebrotendinous xanthomatosis (CTX), congenital adrenal hyperplasia (CAH), megaconial type congenital muscular dystrophy, cerebral creatine deficiency syndrome-3

(CCDS3), maternal nonsyndromic deafness, maternal nonsyndromic deafness, autosomal dominant deafness-64 (DFNA64), Mohr-Tranebjaerg Syndrome, Jensen Syndrome, MEGDEL, reticular dysgenesis, primary coenzyme Q10 deficiency-6 (COQ10D6), CAGSSS, diabetes, Dimethylglycine dehydrogenase deficiency (DMGDHD), Multiple Mitochondrial Dysfunctions Syndrome-1 (MMDS1), Multiple Mitochondrial Dysfunctions Syndrome-2 (MMDS2), Multiple Mitochondrial Dysfunctions Syndrome-3 (MMDS3), childhood leukoencephalopathy associated with mitochondrial Complex II deficiency, encephalopathies associated with mitochondrial Complex I deficiency, encephalopathies associated with mitochondrial Complex III deficiency, encephalopathies associated with mitochondrial Complex IV deficiency, encephalopathies associated with mitochondrial Complex V deficiency, hyperammonemia due to carbonic anhydrase VA deficiency (CA5AD), early infantile epileptic encephalopathy-3 (EIEE3), 2,4-Dienoyl-CoA reductase deficiency (DECRD), infection-induced acute encephalopathy-3 (IIAE3), ethylmalonic encephalopathy (EE), hypomyelinating leukodystrophy (HLD4), exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis, Glutaric aciduria type 1 (GA-1), glycine encephalopathy (GCE), hepatic failure, 2-hydroxyglutaric aciduria, 3-hydroxyacyl-CoA dehydrogenase deficiency, familial hyperinsulinemic hypoglycemia (FHH), hypercalcemia infantile, hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) Syndrome, Immunodeficiency with hyper-IgM type 5 (HIGM5), Inclusion Body Myositis (IBM), polymyositis with mitochondrial pathology, IM-Mito, granulomatous myopathies with anti-mitochondrial antibodies, necrotizing myopathy with pipestem capillaries, myopathy with deficient chondroitin sulfate C in skeletal muscle connective tissue, benign acute childhood myositis, idiopathic orbital myositis, masticator myopathy, hemophagocytic lymphohistiocytosis, infection-associated myositis, Facioscapulohumeral dystrophy (FSH), familial idiopathic inflammatory myopathy, Schmidt Syndrome (Diabetes mellitus, Addison disease, Myxedema), TNF receptor-associated Periodic Syndrome (TRAPS), focal myositis, autoimmune fasciitis, Spanish toxic oil-associated fasciitis, Eosinophilic fasciitis, Macrophagic myofasciitis, Graft-vs-host disease fasciitis, Eosinophilia-myalgia Syndrome, perimyositis, isovaleric acidemia (IVA), Kearnes-Sayre Syndrome (KSS), 2-oxoadipic aciduria, 2-aminoadipic aciduria, Limb-girdle Muscular Dystrophy Syndromes, leukodystrophy, Maple syrup urine disease (MSUD), 3-Methylcrotonyl-CoA carboxylase (MCC), Methylmalonic aciduria (MMA), Miller Syndrome, Mitochondrial DNA Depletion Syndrome-2 (MTDPS2), spinal muscular atrophy syndrome, rigid spine syndrome, severe myopathy with motor regression, Mitochondrial DNA Depletion Syndrome-3, MELAS

Syndrome, camptocormia, MNGIE, MNGIM Syndrome, Menkes Disease, Occipital Horn Syndrome, X-linked distal spinal muscular atrophy-3 (SMAX3), methemoglobinemia, MERRF, progressive external ophthalmoplegia with myoclonus, deafness and diabetes (DD), multiple symmetric lipomatosis, Myopathy with Episodic high Creatine Kinase (MIMECK), Epilepsia Partialis Continua, malignant hyperthermia syndromes, glycogen metabolic disorders, fatty acid oxidation and lipid metabolism disorders, medication-, drug- or toxin-induced myoglobinuria, mitochondrial disorder-associated myoglobinuria, hypokalemic myopathy and rhabdomyolysis, muscle trauma-associated myoglobinuria, ischemia-induced myoglobinuria, infection-induced myoglobinuria, immune myopathies associated with myoglobinuria, Myopathy, lactic acidosis, and sideroblastic anemia (MLASA), infantile mitochondrial myopathy due to reversible COX deficiency (MMIT), Myopathy, Exercise intolerance, Encephalopathy and Lactic acidemia Syndrome, myoglobinuria and exercise intolerance syndrome, exercise intolerance, proximal weakness ± myoglobinuria syndrome, encephalopathy and seizures syndrome, septo-optic dysplasia, exercise intolerance ± mild weakness, myopathy ± exercise intolerance, growth or CNS disorder, maternally-inherited mitochondrial myopathies, myopathy with lactic acidosis, myopathy with rhabdomyolysis, Myopathy with cataract and combined respiratory chain deficiency, myopathy with abnormal mitochondrial translation, Fatigue Syndrome, myopathy with extrapyramidal movement disorders (MPXPS), glutaric aciduria II (MADD), primary CoQ10 deficiency-1 (COQ10D1), primary CoQ10 deficiency-2 (COQ10D2), primary CoQ10 deficiency-3 (COQ10D3), primary CoQ10 deficiency-5 (COQ10D5), secondary CoQ10 deficiency, autosomal dominant mitochondrial myopathy, myopathy with focal depletion of mitochondria, mitochondrial DNA breakage syndrome (PEO + Myopathy), lipid type mitochondrial myopathy, multiple symmetric lipomatosis (MSL), N-acetylglutamate synthase (NAGS) deficiency, Nephronophthisis (NPHP), ornithine transcarbamylase (OTC) deficiency, neoplasms, NARP Syndrome, paroxysmal nonkinesigenic dyskinesia (PNKD), sporadic PEO, maternally-inherited PEO, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-3 (PEOA3), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-2 (PEOA2), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-1 (PEOA1), PEO+ demyelinating neuropathy, PEO + hypogonadism, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-4 (PEOA4), distal myopathy, cachexia & PEO, autosomal dominant progressive external ophthalmoplegia-6 (PEOA6), PEO + Myopathy and Parkinsonism, autosomal recessive progressive external ophthalmoplegia

(PEOB), Mitochondrial DNA Depletion Syndrome-11 (MTDPS11), PEO with cardiomyopathy, PEPCK deficiency, Perrault Syndromes (PRLTS), propionic acidemia (PA), pyruvate carboxylase deficiency, pyruvate dehydrogenase E1-alpha deficiency (PDHAD), pyruvate dehydrogenase E1-beta deficiency (PDHBD), dihydrolipoamide dehydrogenase (DLD) deficiency, pyruvate dehydrogenase phosphatase deficiency, pyruvate dehydrogenase E3-binding protein deficiency (PDHXD), mitochondrial pyruvate carrier deficiency (MPYCD), Schwartz-Jampel Syndrome type 1 (SJS1), selenium deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency, succinyl CoA:3-oxacid CoA transferase (SCOT) deficiency, Stuve-Wiedemann Syndrome (STWS), thrombocytopenia (THC), Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), Vitamin D-dependent rickets type 1A (VDDR1A), Wilson's disease, Zellweger Syndrome (PBD3A), arsenic trioxide myopathy, myopathy and neuropathy resulting from nucleoside analogues, germanium myopathy, Parkinsonism and mitochondrial Complex I neurotoxicity due to trichloroethylene, valproate-induced hepatic failure, neurodegeneration with brain iron accumulation-4 (NBIA4), Complex I deficiency, Complex II deficiency, Complex III deficiency, Complex IV deficiency, Complex V deficiency, Cytochrome c oxidase (COX) deficiency, combined complex I, II, IV, ± V deficiency, combined complex I, II, and III deficiency, combined oxidative phosphorylation deficiency-1 (COXPD1), combined oxidative phosphorylation deficiency-2 (COXPD2), combined oxidative phosphorylation deficiency-3 (COXPD3), combined oxidative phosphorylation deficiency-4 (COXPD4), combined oxidative phosphorylation deficiency-6 (COXPD6), combined oxidative phosphorylation deficiency-7 (COXPD7), combined oxidative phosphorylation deficiency-9 (COXPD9), combined oxidative phosphorylation deficiency-11 (COXPD11), combined oxidative phosphorylation deficiency-12 (COXPD12), combined oxidative phosphorylation deficiency-13 (COXPD13), combined oxidative phosphorylation deficiency-15 (COXPD15), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-19 (COXPD19), combined oxidative phosphorylation deficiency-20 (COXPD20), combined oxidative phosphorylation deficiency-21 (COXPD21), fumarase deficiency, HMG-CoA synthase-2 deficiency, hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (HUPRA) Syndrome, syndromic microphthalmia-7, pontocerebellar hypoplasia type 6 (PCH6), Mitochondrial DNA Depletion Syndrome-9 (MTDPS9P), and Sudden infant death Syndrome (SIDS).

[0463] In one aspect, the present disclosure provides a method of treating a disease or condition characterized by excessive mitochondrial fission, comprising administering a therapeutically effective amount of a composition comprising mitochondrial-targeted creatine compounds, or derivatives, analogues, or pharmaceutically acceptable salts thereof.

[0464] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) are useful to regulate mitochondrial fission.

[0465] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful to prevent or treat Leber's Hereditary Optic Neuropathy.

[0466] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful to prevent or treat Friedreich's Ataxia.

[0467] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful to treat one or more signs, symptoms or complications of Friedreich's Ataxia including mitochondrial iron loading, Complex I and ATP content deficiency, and defects in iron-sulfur cluster biosynthesis.

[0468] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful to reduce tumor growth in a subject in need thereof.

Treatment of a Mitochondrial Disease or Disorder

[0469] In some embodiments, the disclosure provides for both prophylactic and therapeutic methods of treating a subject having or suspected of having a mitochondrial disease, condition or disorder. For example, in some embodiments, the disclosure provides for both prophylactic and therapeutic methods of treating a subject having a disruption in oxidative phosphorylation caused by a gene mutation *e.g.*, SURF1, POLG *etc.*

[0470] In some embodiments, the present technology provides methods for the treatment, amelioration or prevention of a mitochondrial disease, condition or disorder in subjects

through administration of therapeutically effective amounts of mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology as disclosed herein to subjects in need thereof. In some embodiments of the method, the mitochondrial disease, condition or disorder is selected from the group consisting of Alexander disease, Alpers Syndrome, Alpha-ketoglutarate dehydrogenase (AKDGH) deficiency, ALS-FTD, Sideroblastic anemia with spinocerebellar ataxia, Pyridoxine-refractory sideroblastic anemia, GRACILE Syndrome, Björnstad Syndrome, Leigh Syndrome, mitochondrial complex III deficiency nuclear type 1 (MC3DN1), combined oxidative phosphorylation deficiency 18 (COXPD18), Thiamine-responsive megaloblastic anemia syndrome (TRMA), Pearson Syndrome, HAM Syndrome, Ataxia, Cataract, and Diabetes Syndrome, MELAS/MERRF Overlap Syndrome, combined oxidative phosphorylation deficiency-14 (COXPD14), Infantile cerebellar-retinal degeneration (ICRD), Charlevoix-Saguenay spastic ataxia, Primary coenzyme Q10 deficiency-1 (COQ10D1), ataxia oculomotor apraxia type 1 (AOA1), Autosomal recessive spinocerebellar ataxia-9/ coenzyme Q10 deficiency-4 (COQ10D4), Ataxia, Pyramidal Syndrome, and Cytochrome Oxidase Deficiency, Friedreich's ataxia, Infantile onset spinocerebellar ataxia (IOSCA)/ Mitochondrial DNA Depletion Syndrome-7, Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), Autosomal recessive spastic ataxia-3 (SPAX3), MIRAS, SANDO, mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), spastic ataxia with optic atrophy (SPAX4), progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5), mitochondrial complex III deficiency nuclear type 2 (MC3DN2), episodic encephalopathy due to thiamine pyrophosphokinase deficiency/Thiamine Metabolism Dysfunction Syndrome-5 (THMD5), Spinocerebellar ataxia-28 (SCA28), autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCA-DN), Dominant Optic Atrophy (DOA), cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) Syndrome, spinocerebellar ataxia 7 (SCA7), Barth Syndrome, Biotinidase deficiency, gyrate atrophy, Syndromic Dominant Optic Atrophy and Deafness (Syndromic DOAD), Dominant Optic Atrophy plus (DOAplus), Leber's hereditary optic neuropathy (LHON), Wolfram Syndrome-1 (WFS1), Wolfram Syndrome-2 (WFS2), Age-related macular degeneration (ARMD), Brunner Syndrome, Left ventricular noncompaction-1 (LVNC1), histiocytoid cardiomyopathy, Familial Myalgia Syndrome, Parkinsonism, Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency-1 (CEMCOX1), Sengers Syndrome, Cardiofaciocutaneous Syndrome-1 (CFC1), Mitochondrial

trifunctional protein (MTP) deficiency, infantile encephalocardiomyopathy with cytochrome c oxidase deficiency, cardiomyopathy + encephalomyopathy, mitochondrial phosphate carrier deficiency, infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency (CEMCOX2), β-Hydroxyisobutyryl CoA Deacylase (HIBCH) deficiency, ECHS1) deficiency, Maternal Inheritance Leigh Syndrome (MILS), dilated cardiomyopathy with ataxia (DCMA), Mitochondrial DNA Depletion Syndrome-12 (MTDPS12), cardiomyopathy due to mitochondrial tRNA deficiencies, mitochondrial complex V (ATP synthase) deficiency nuclear type 1 (MC5DN1), combined oxidative phosphorylation deficiency-8 (COXPD8), progressive leukoencephalopathy with ovarian failure (LKENP), combined oxidative phosphorylation deficiency-10 (COXPD10), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-17 (COXPD17), combined oxidative phosphorylation deficiency-5 (COXPD5), combined oxidative phosphorylation deficiency-9 (COXPD9), carnitine acetyltransferase (CRAT) deficiency, carnitine palmitoyltransferase I (CPT I) deficiency, myopathic carnitine deficiency, primary systemic carnitine deficiency (CDSP), carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acylcarnitine translocase deficiency (CACTD), cartilage-hair hypoplasia, cerebrotendinous xanthomatosis (CTX), congenital adrenal hyperplasia (CAH), megaconial type congenital muscular dystrophy, cerebral creatine deficiency syndrome-3 (CCDS3), maternal nonsyndromic deafness, maternal nonsyndromic deafness, autosomal dominant deafness-64 (DFNA64), Mohr-Tranebjaerg Syndrome, Jensen Syndrome, MEGDEL, reticular dysgenesis, primary coenzyme Q10 deficiency-6 (COQ10D6), CAGSSS, diabetes, Dimethylglycine dehydrogenase deficiency (DMGDHD), Multiple Mitochondrial Dysfunctions Syndrome-1 (MMDS1), Multiple Mitochondrial Dysfunctions Syndrome-2 (MMDS2), Multiple Mitochondrial Dysfunctions Syndrome-3 (MMDS3), childhood leukoencephalopathy associated with mitochondrial Complex II deficiency, encephalopathies associated with mitochondrial Complex I deficiency, encephalopathies associated with mitochondrial Complex III deficiency, encephalopathies associated with mitochondrial Complex IV deficiency, encephalopathies associated with mitochondrial Complex V deficiency, hyperammonemia due to carbonic anhydrase VA deficiency (CA5AD), early infantile epileptic encephalopathy-3 (EIEE3), 2,4-Dienoyl-CoA reductase deficiency (DECRD), infection-induced acute encephalopathy-3 (IIAE3), ethylmalonic encephalopathy (EE), hypomyelinating leukodystrophy (HLD4), exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis, Glutaric aciduria type 1 (GA-1), glycine encephalopathy (GCE), hepatic failure, 2-hydroxyglutaric aciduria, 3-hydroxyacyl-CoA

dehydrogenase deficiency, familial hyperinsulinemic hypoglycemia (FHH), hypercalcemia infantile, hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) Syndrome, Immunodeficiency with hyper-IgM type 5 (HIGM5), Inclusion Body Myositis (IBM), polymyositis with mitochondrial pathology, IM-Mito, granulomatous myopathies with anti-mitochondrial antibodies, necrotizing myopathy with pipestem capillaries, myopathy with deficient chondroitin sulfate C in skeletal muscle connective tissue, benign acute childhood myositis, idiopathic orbital myositis, masticator myopathy, hemophagocytic lymphohistiocytosis, infection-associated myositis, Facioscapulohumeral dystrophy (FSH), familial idiopathic inflammatory myopathy, Schmidt Syndrome (Diabetes mellitus, Addison disease, Myxedema), TNF receptor-associated Periodic Syndrome (TRAPS), focal myositis, autoimmune fasciitis, Spanish toxic oil-associated fasciitis, Eosinophilic fasciitis, Macrophagic myofasciitis, Graft-vs-host disease fasciitis, Eosinophilia-myalgia Syndrome, perimyositis, isovaleric acidemia (IVA), Kearnes-Sayre Syndrome (KSS), 2-oxoadipic aciduria, 2-aminoadipic aciduria, Limb-girdle Muscular Dystrophy Syndromes, leukodystrophy, Maple syrup urine disease (MSUD), 3-Methylcrotonyl-CoA carboxylase (MCC), Methylmalonic aciduria (MMA), Miller Syndrome, Mitochondrial DNA Depletion Syndrome-2 (MTDPS2), spinal muscular atrophy syndrome, rigid spine syndrome, severe myopathy with motor regression, Mitochondrial DNA Depletion Syndrome-3, MELAS Syndrome, camptocormia, MNGIE, MNGIM Syndrome, Menkes Disease, Occipital Horn Syndrome, X-linked distal spinal muscular atrophy-3 (SMAX3), methemoglobinemia, MERRF, progressive external ophthalmoplegia with myoclonus, deafness and diabetes (DD), multiple symmetric lipomatosis, Myopathy with Episodic high Creatine Kinase (MIMECK), Epilepsia Partialis Continua, malignant hyperthermia syndromes, glycogen metabolic disorders, fatty acid oxidation and lipid metabolism disorders, medication-, drug- or toxin-induced myoglobinuria, mitochondrial disorder-associated myoglobinuria, hypokalemic myopathy and rhabdomyolysis, muscle trauma-associated myoglobinuria, ischemia-induced myoglobinuria, infection-induced myoglobinuria, immune myopathies associated with myoglobinuria, Myopathy, lactic acidosis, and sideroblastic anemia (MLASA), infantile mitochondrial myopathy due to reversible COX deficiency (MMIT), Myopathy, Exercise intolerance, Encephalopathy and Lactic acidemia Syndrome, myoglobinuria and exercise intolerance syndrome, exercise intolerance, proximal weakness ± myoglobinuria syndrome, encephalopathy and seizures syndrome, septo-optic dysplasia, exercise intolerance ± mild weakness, myopathy ± exercise intolerance, growth or CNS disorder, maternally-inherited mitochondrial myopathies, myopathy with lactic acidosis, myopathy with rhabdomyolysis,

Myopathy with cataract and combined respiratory chain deficiency, myopathy with abnormal mitochondrial translation, Fatigue Syndrome, myopathy with extrapyramidal movement disorders (MPXPS), glutaric aciduria II (MADD), primary CoQ10 deficiency-1 (COQ10D1), primary CoQ10 deficiency-2 (COQ10D2), primary CoQ10 deficiency-3 (COQ10D3), primary CoQ10 deficiency-5 (COQ10D5), secondary CoQ10 deficiency, autosomal dominant mitochondrial myopathy, myopathy with focal depletion of mitochondria, mitochondrial DNA breakage syndrome (PEO + Myopathy), lipid type mitochondrial myopathy, multiple symmetric lipomatosis (MSL), N-acetylglutamate synthase (NAGS) deficiency, Nephronophthisis (NPHP), ornithine transcarbamylase (OTC) deficiency, neoplasms, NARP Syndrome, paroxysmal nonkinesigenic dyskinesia (PNKD), sporadic PEO, maternally-inherited PEO, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-3 (PEOA3), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-2 (PEOA2), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-1 (PEOA1), PEO+ demyelinating neuropathy, PEO + hypogonadism, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-4 (PEOA4), distal myopathy, cachexia & PEO, autosomal dominant progressive external ophthalmoplegia-6 (PEOA6), PEO + Myopathy and Parkinsonism, autosomal recessive progressive external ophthalmoplegia (PEOB), Mitochondrial DNA Depletion Syndrome-11 (MTDPS11), PEO with cardiomyopathy, PEPCK deficiency, Perrault Syndromes (PRLTS), propionic acidemia (PA), pyruvate carboxylase deficiency, pyruvate dehydrogenase E1-alpha deficiency (PDHAD), pyruvate dehydrogenase E1-beta deficiency (PDHBD), dihydrolipoamide dehydrogenase (DLD) deficiency, pyruvate dehydrogenase phosphatase deficiency, pyruvate dehydrogenase E3-binding protein deficiency (PDHXD), mitochondrial pyruvate carrier deficiency (MPYCD), Schwartz-Jampel Syndrome type 1 (SJS1), selenium deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency, succinyl CoA:3-oxacid CoA transferase (SCOT) deficiency, Stuve-Wiedemann Syndrome (STWS), thrombocytopenia (THC), Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), Vitamin D-dependent rickets type 1A (VDDR1A), Wilson's disease, Zellweger Syndrome (PBD3A), arsenic trioxide myopathy, myopathy and neuropathy resulting from nucleoside analogues, germanium myopathy, Parkinsonism and mitochondrial Complex I neurotoxicity due to trichloroethylene, valproate-induced hepatic failure, neurodegeneration with brain iron accumulation-4 (NBIA4), Complex I deficiency, Complex II deficiency, Complex III deficiency, Complex IV deficiency, Complex V deficiency, Cytochrome c oxidase (COX) deficiency, combined

complex I, II, IV, ± V deficiency, combined complex I, II, and III deficiency, combined oxidative phosphorylation deficiency-1 (COXPD1), combined oxidative phosphorylation deficiency-2 (COXPD2), combined oxidative phosphorylation deficiency-3 (COXPD3), combined oxidative phosphorylation deficiency-4 (COXPD4), combined oxidative phosphorylation deficiency-6 (COXPD6), combined oxidative phosphorylation deficiency-7 (COXPD7), combined oxidative phosphorylation deficiency-9 (COXPD9), combined oxidative phosphorylation deficiency-11 (COXPD11), combined oxidative phosphorylation deficiency-12 (COXPD12), combined oxidative phosphorylation deficiency-13 (COXPD13), combined oxidative phosphorylation deficiency-15 (COXPD15), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-19 (COXPD19), combined oxidative phosphorylation deficiency-20 (COXPD20), combined oxidative phosphorylation deficiency-21 (COXPD21), fumarase deficiency, HMG-CoA synthase-2 deficiency, hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (HUPRA) Syndrome, syndromic microphthalmia-7, pontocerebellar hypoplasia type 6 (PCH6), Mitochondrial DNA Depletion Syndrome-9 (MTDPS9P), and Sudden infant death Syndrome (SIDS).

[0471] In some embodiments, the present technology provides methods for the prevention and/or treatment of mitochondrial disease or disorder in a subject by administering an effective amount of mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology to a subject in need thereof to reduce disruption in oxidative phosphorylation of the subject.

[0472] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in decreasing intracellular ROS (reactive oxygen species) and increasing survival in cells of a subject in need thereof, *e.g.*, a subject suffering from a disease or condition characterized by mitochondrial dysfunction.

[0473] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in preventing loss of cell viability in subjects suffering from a disease or condition characterized by mitochondrial dysfunction.

[0474] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in decreasing the percent of cells showing increased caspase activity in a subject in need thereof, *e.g.*, a subject suffering from a disease or condition characterized by mitochondrial dysfunction.

[0475] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in reducing the rate of ROS accumulation in a subject in need thereof, *e.g.*, a subject suffering from a disease or condition characterized by mitochondrial dysfunction.

[0476] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in inhibiting lipid peroxidation in a subject in need thereof, *e.g.*, a subject suffering from a disease or condition characterized by mitochondrial dysfunction.

[0477] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in preventing mitochondrial depolarization and ROS accumulation in a subject in need thereof, *e.g.*, a subject suffering from a disease or condition characterized by mitochondrial dysfunction.

[0478] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in preventing apoptosis in a subject in need thereof, *e.g.*, a subject suffering from a disease or condition characterized by mitochondrial dysfunction.

[0479] In one aspect, the present technology provides a method of preventing, treating or ameliorating a medical disease or condition by administering a therapeutically effective amount of mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) to a subject in need thereof. In some embodiments the medical disease or condition is a mitochondrial disorder. In another aspect, the present technology provides a method of modulating one or more energy biomarkers, normalizing one or more energy biomarkers, or enhancing one or more energy biomarkers by administering a therapeutically effective amount of mitochondrial-targeted creatine

compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) to a subject in need thereof.

Use of Mitochondrial-Targeted Creatine Compounds to Treat IPF, Alport Syndrome, Vitiligo, and Porphyria

[0480] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in methods for treating, ameliorating, or reversing idiopathic pulmonary fibrosis (IPF). In certain embodiments of the method, IPF is induced by TGF-β signaling. In other embodiments of the method, IPF is induced by exposure to bleomycin. In some embodiments of the methods, IPF symptoms or signs include an increase in TGF-β1-induced epithelial to mesenchymal transition (EMT), myofibroblast activation, collagen production, and severe progressive fibrosis including fibrotic foci and honeycombing. In some embodiments, EMT is characterized by loss of epithelial markers such as E-cadherin, cytoskeletal reorganization, and transition to a spindle-shaped morphology with the acquisition of mesenchymal markers (α-SMA and collagen I). EMT of alveolar epithelial cells (AECs) has been widely observed in patients with IPF.

[0481] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in reducing the formation of fibroblast foci and lung scarring in the subject, as evidenced by a decrease in collagen content using sircol assay and fibrosis scoring.

[0482] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in reducing myofibroblast activation and collagen production in the subject, as evidenced by a decrease in α-SMA and collagen I expression.

[0483] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in reducing TGF-β1-induced EMT in the subject, as evidenced by the persistence of E-cadherin expression and/or decrease in spindle-shaped morphology. In some embodiments, administration of a therapeutically effective dose of mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) to a subject in need thereof, results in a reduction of TGF-β1-induced EMT in the subject, as

evidenced by a decrease in α-SMA or vimentin expression. In some embodiments, administration of a therapeutically effective dose of mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) to a subject in need thereof, results in a reduction of TGF-β1-induced EMT in the subject, as evidenced by a decrease in cytoskeletal reorganization.

[0484] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in treating or ameliorating melanocyte degeneration in a subject in need thereof. In one particular embodiment, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in the treatment, amelioration or prevention of vitiligo in subjects through administration of therapeutically effective amounts of mitochondrial-targeted creatine compounds as disclosed herein, or pharmaceutically acceptable salts thereof, to subjects in need thereof.

[0485] Vitiligo is a pigmentation disorder in which melanocytes, the cells responsible for skin pigmentation, are destroyed. As a result, white patches appear on the skin in different parts of the body. Vitiligo lesions can appear anywhere, but are most commonly found on the acral areas, mucous membranes (tissues that line the inside of the mouth and nose), retina and genitals. Other symptoms include increased photosensitivity, decreased contact sensitivity response to dinitrochlorobenzene, and premature whitening or graying of hair that grows on areas affected by vitiligo. Non-segmental vitiligo (NSV) is associated with some form of symmetry in the location of the patches of depigmentation. Classes of NSV include generalized vitiligo, universal vitiligo, and focal vitiligo. Generalized vitiligo (GV), the most common category, affects approximately 0.5% of the world's population, with an average age of onset at about 24 years and occurring with approximately equal frequencies in males and females. Vitiligo lesions have an infiltrate of inflammatory cells, particularly cytotoxic and helper T cells and macrophages. Patients with vitiligo are also more likely to have at least one other autoimmune disease including Hashimoto's thyroiditis, Graves' disease, pernicious anemia, rheumatoid arthritis, psoriasis, type I diabetes, Addison's disease, celiac disease, inflammatory bowel disorder, and systemic lupus erythematosus.

[0486] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in the treatment, amelioration or prevention of porphyria in a subject in need thereof. The

porphyrias, are metabolic disorders, each resulting from the deficiency of a specific enzyme in the heme biosynthetic pathway. These enzyme deficiencies are inherited as autosomal dominant, autosomal recessive, or X-linked traits, with the exception of the most common porphyria, porphyria cutanea tarda, which usually is sporadic. Porphyrias have been classified as either hepatic or erythropoietic depending on the primary site of overproduction and accumulation of porphyrin precursors or porphyrins, although some porphyrias have overlapping features. The hepatic porphyrias are characterized by overproduction and initial accumulation of the porphyrin precursors, ALA and PBG, and/or porphyrins primarily in the liver, whereas in the erythropoietic porphyrias, overproduction and initial accumulation of the pathway intermediates occur primarily in bone marrow erythroid cells. The eight major porphyrias can be classified into three groups: (1) the four acute hepatic porphyrias, (2) the single hepatic cutaneous porphyria (*i.e.*, porphyria cutanea tarda), and (3) the three erythropoietic cutaneous porphyrias. In certain embodiments, the acute hepatic porphyria is acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) or autosomal recessive ALA-dehydratase-deficient porphyria. In some embodiments, the erythropoietic cutaneous porphyria is congenital erythropoietic porphyria (CEP), erythropoietic protoporphyria (EPP) and X-linked porphyria (XLP). Symptoms associated with porphyria include, but are not limited to, cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, life threatening acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension.

[0487] Variegate porphyria (VP) is an autosomal dominant disorder of porphyrin-heme metabolism characterized by accumulations of the photosensitizing porphyrins, protoporphyrin and coproporphyrin, arising from mutations of the gene encoding the enzyme protoporphyrinogen oxidase (PPOX). PPOX is an enzyme in the heme biosynthetic pathway that catalyzes the oxidation of protoporphyrinogen IX to form protoporphyrin IX. PPOX is localized to the mitochondrial intermembrane space and is found in various tissues, including liver, lymphocytes, and cultured fibroblasts.

[0488] Manifestations of VP may include cutaneous manifestations, including photosensitivity, blistering, skin fragility, and postinflammatory hyperpigmentation. Acute exacerbations of VP are characterized by the occurrence of neuro-visceral attacks that include abdominal pain, the passage of dark urine, and neuropsychiatric symptoms such as bulbar paralysis, quadriplegia, motor neuropathy, and weakness of the limbs. VP is associated with a heterozygous mutation in the gene for protoporphyrinogen oxidase (PPOX). The homozygous variant of VP is characterized by severe PPOX deficiency, onset of photosensitization by porphyrins in early childhood, skeletal abnormalities of the hand, short stature, mental retardation, and convulsions.

[0489] In some embodiments, mitochondrial-targeted creatine compounds (or derivatives, analogues, or pharmaceutically acceptable salts thereof) of the present technology are useful in the treatment, amelioration or prevention of Alport Syndrome in a subject in need thereof. Alport Syndrome is a genetic condition characterized by kidney disease, hearing loss, and eye abnormalities and occurs in approximately 1 in 50,000 newborns. Mutations in the COL4A3, COL4A4, and COL4A5 genes cause Alport Syndrome. These genes each provide instructions for making one component of a protein called type IV collagen. This protein plays an important role in the kidneys, specifically in structures called glomeruli. Glomeruli are clusters of specialized blood vessels that remove water and waste products from blood and create urine. Mutations in these genes result in abnormalities of the type IV collagen in glomeruli, which prevents the kidneys from properly filtering the blood and allows blood and protein to pass into the urine. Gradual scarring of the kidneys occurs, eventually leading to progressive loss of kidney function and end-stage renal disease in many people with Alport Syndrome.

[0490] Type IV collagen is also an important component of inner ear structures, particularly the organ of Corti, that transform sound waves into nerve impulses for the brain. Alterations in type IV collagen often result in abnormal inner ear function during late childhood or early adolescence, which can lead to sensorineural deafness. In the eye, type IV collagen is important for maintaining the shape of the lens and the normal color of the retina. Mutations that disrupt type IV collagen can result in misshapen lenses (anterior lenticonus) and an abnormally colored retina. Significant hearing loss, eye abnormalities, and progressive kidney disease are more common in males with Alport Syndrome than in affected females. Symptoms associated with Alport Syndrome, including, but not limited to, *e.g.*, hematuria,

proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, Glomerular Basement Membrane (GBM) ultrastructural abnormalities, nephrotic Syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis.

Therapeutic Methods

[0491] The following discussion is presented by way of example only, and is not intended to be limiting.

[0492] One aspect of the present technology includes methods of treating a mitochondrial disease or disorder in a subject diagnosed as having, suspected as having, or at risk of having a mitochondrial disease or disorder. In therapeutic applications, compositions or medicaments comprising a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, are administered to a subject suspected of, or already suffering from such a disease (such as, *e.g.*, subjects exhibiting pathological levels of one or more energy biomarkers such as, lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; total, reduced or oxidized glutathione levels; total, reduced or oxidized cysteine levels; phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; acetoacetate levels; beta-hydroxy butyrate levels; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; and reactive oxygen species levels compared to a normal control subject, or alternatively a subject diagnosed with a mitochondrial disease or disorder), in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease, including its complications and intermediate pathological phenotypes in development of the disease. In some embodiments of the method, the lactate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the pyruvate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized glutathione levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid,

or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized cysteine levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject.

[0493] Subjects suffering from a mitochondrial disease or disorder can be identified by any or a combination of diagnostic or prognostic assays known in the art. For example, typical symptoms of a mitochondrial disease or disorder include, but are not limited to, poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, brain atrophy, or any other sign or symptom of a mitochondrial disease state disclosed herein.

[0494] In some embodiments, the subject may exhibit pathological levels of one or more energy biomarkers such as, lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; total, reduced or oxidized glutathione levels; total, reduced or oxidized cysteine levels; phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; acetoacetate levels; beta-hydroxy butyrate levels; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; and reactive oxygen species levels compared to a normal control subject, which is measureable using techniques known in the art. In some embodiments of the method, the lactate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the pyruvate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some embodiments of the method, the total, reduced or oxidized glutathione levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject. In some

embodiments of the method, the total, reduced or oxidized cysteine levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid are abnormal compared to a normal control subject.

[0495] In some embodiments, the subject may exhibit one or more mtDNA or nuclear DNA mutations in one or more genes described herein that play a biological/physiological role in the mitochondria (*e.g.*, mitochondrial protein synthesis, respiratory chain function, intergenomic signaling, mitochondrial importation of nDNA-encoded proteins, synthesis of inner mitochondrial membrane phospholipids, mitochondrial motility and fission, mitophagy *etc.*). Such mutations are detectable using techniques known in the art.

[0496] In some embodiments, administration of one or more mitochondrial-targeted creatine compounds to subjects suffering from a mitochondrial disease or disorder will result in the amelioration or elimination of one or more of the following symptoms: poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, brain atrophy, or any other sign or symptom of a mitochondrial disease state disclosed herein.

[0497] In some embodiments, administration of one or more mitochondrial-targeted creatine compounds to subjects suffering from a mitochondrial disease or disorder will result in the normalization of one or more energy biomarkers such as, lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; total, reduced or oxidized glutathione levels; total, reduced or oxidized cysteine levels; phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; acetoacetate levels; beta-hydroxy butyrate levels; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; and reactive oxygen species levels compared to untreated subjects with a mitochondrial disease or disorder. In some embodiments of the method, the lactate levels of one or more of whole blood, plasma,

cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are normalized compared to untreated subjects with a mitochondrial disease or disorder. In some embodiments of the method, the pyruvate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are normalized compared to untreated subjects with a mitochondrial disease or disorder. In some embodiments of the method, the total, reduced or oxidized glutathione levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are normalized compared to untreated subjects with a mitochondrial disease or disorder. In some embodiments of the method, the total, reduced or oxidized cysteine levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are normalized compared to untreated subjects with a mitochondrial disease or disorder.

[0498] One aspect of the present technology includes methods of treating Alport Syndrome in a subject diagnosed as having, suspected as having, or at risk of having Alport Syndrome. In therapeutic applications, compositions or medicaments comprising a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, are administered to a subject suspected of, or already suffering from such a disease (such as, *e.g.*, subjects exhibiting aberrant levels and/or function of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin compared to a normal control subject, or a subject diagnosed with Alport Syndrome), in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease, including its complications and intermediate pathological phenotypes in development of the disease.

[0499] Subjects suffering from Alport Syndrome can be identified by any or a combination of diagnostic or prognostic assays known in the art. For example, typical symptoms of Alport Syndrome include, but are not limited to, hematuria, proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, GBM ultrastructural abnormalities, nephrotic syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis.

[0500] In some embodiments, the subject may exhibit aberrant levels or function of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin compared to a

normal control subject, which is measureable using techniques known in the art. In some embodiments, the subject may exhibit one or more mutations in COL4A3, COL4A4, and COL4A5, which are involved in the production or assembly of type IV collagen fibers and are detectable using techniques known in the art.

[0501] In some embodiments, Alport Syndrome subjects treated with one or more mitochondrial-targeted creatine compounds will show amelioration or elimination of one or more of the following symptoms: hematuria, proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, GBM ultrastructural abnormalities, nephrotic syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis. In certain embodiments, Alport Syndrome subjects treated with one or more mitochondrial-targeted creatine compounds will show normalization of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin urine levels by at least 10% compared to untreated Alport Syndrome subjects. In certain embodiments, Alport Syndrome subjects treated with one or more mitochondrial-targeted creatine compounds will show MMP-9 expression levels in mesangial cells that are similar to that observed in a normal control subject.

[0502] In another aspect, the present technology includes methods of treating porphyria in a subject diagnosed as having, suspected as having, or at risk of having porphyria. In therapeutic applications, compositions or medicaments comprising a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, are administered to a subject suspected of, or already suffering from such a disease, such as, *e.g.*, aberrant levels and/or function of enzymes involved in heme biosynthesis compared to a normal control subject or porphyria, in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease, including its complications and intermediate pathological phenotypes in development of the disease.

[0503] Subjects suffering from porphyria can be identified by any or a combination of diagnostic or prognostic assays known in the art. For example, typical symptoms of porphyria include, but are not limited to, cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the

skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, life threatening acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension.

[0504] In some embodiments, the subject may exhibit aberrant levels or function of enzymes required for heme biosynthesis compared to a normal control subject, which is measureable using techniques known in the art. In some embodiments, the subject may exhibit one or more mutations in genes encoding enzymes required for heme biosynthesis, which are detectable using techniques known in the art.

[0505] In some embodiments, administration of one or more mitochondrial-targeted creatine compounds to a subject that is diagnosed as having, is suspected of having, or is at risk of having porphyria will result in amelioration or elimination of one or more of the following symptoms: cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, life threatening acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension. In certain embodiments of the method, porphyria subjects treated with the mitochondrial-targeted creatine compound will show normalization of the levels and/or function of one or more enzymes required for heme biosynthesis compared to untreated porphyria subjects.

[0506] One aspect of the present technology includes methods of treating vitiligo in a subject diagnosed as having, suspected as having, or at risk of having vitiligo. In therapeutic applications, compositions or medicaments comprising a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, are administered to a subject suspected of, or already suffering from such a disease, in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease, including its complications and intermediate pathological phenotypes in development of the disease.

[0507] Subjects suffering from vitiligo can be identified by any or a combination of diagnostic or prognostic assays known in the art. For example, typical symptoms of vitiligo include, but are not limited to, increased photosensitivity, decreased contact sensitivity response to dinitrochlorobenzene, depigmentation of the skin, mucous membranes (tissues that line the inside of the mouth and nose), retina, or genitals, and premature whitening or graying of hair on the scalp, eyelashes, eyebrows or beard.

[0508] In some embodiments, vitiligo subjects treated with one or more mitochondrial-targeted creatine compounds will show amelioration or elimination of one or more of the following symptoms: increased photosensitivity, decreased contact sensitivity response to dinitrochlorobenzene, depigmentation of the skin, mucous membranes (tissues that line the inside of the mouth and nose), retina, or genitals, and premature whitening or graying of hair on the scalp, eyelashes, eyebrows or beard.

[0509] In some embodiments, administration of one or more mitochondrial-targeted creatine compounds to a subject that is diagnosed as having, is suspected of having, or is at risk of having IPF will result in amelioration or elimination of one or more of the following symptoms: increase in TGF-β1-induced epithelial to mesenchymal transition (EMT), myofibroblast activation, collagen production, lung scarring, and severe progressive fibrosis including fibrotic foci and honeycombing.

Prophylactic Methods

[0510] In one aspect, the present technology provides a method for preventing or delaying the onset of a mitochondrial disease or disorder in a subject at risk of having a mitochondrial disease or disorder. In some embodiments, the subject may exhibit one or more mtDNA or nuclear DNA mutations in one or more genes described herein that play a biological/physiological role in the mitochondria (*e.g.*, mitochondrial protein synthesis, respiratory chain function, intergenomic signaling, mitochondrial importation of nDNA-encoded proteins, synthesis of inner mitochondrial membrane phospholipids, mitochondrial motility and fission, mitophagy *etc.*).

[0511] Subjects at risk for pathological levels of one or more energy biomarkers such as, lactic acid (lactate) levels (in one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid); pyruvic acid (pyruvate) levels (in one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid); total, reduced or oxidized

glutathione levels (in one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid); total, reduced or oxidized cysteine levels (in one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid); phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; acetoacetate levels; beta-hydroxy butyrate levels; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; and reactive oxygen species levels compared to a normal control subject, or alternatively a mitochondrial disease or disorder, can be identified by, *e.g.*, any or a combination of diagnostic or prognostic assays known in the art.

[0512] In prophylactic applications, pharmaceutical compositions or medicaments comprising a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, are administered to a subject susceptible to, or otherwise at risk of a mitochondrial disease or disorder in an amount sufficient to eliminate or reduce the risk, or delay the onset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Administration of a prophylactic mitochondrial-targeted creatine compound can occur prior to the manifestation of symptoms characteristic of the disease or disorder, such that the disease or disorder is prevented or, alternatively, delayed in its progression.

[0513] Subjects at risk for pathological levels of one or more energy biomarkers compared to a normal control subject, or alternatively a mitochondrial disease or disorder include, but are not limited to, subjects harboring mutations in one or more genes described herein that play a biological/physiological role in the mitochondria (*e.g.*, mitochondrial protein synthesis, respiratory chain function, intergenomic signaling, mitochondrial importation of nDNA-encoded proteins, synthesis of inner mitochondrial membrane phospholipids, mitochondrial motility and fission, mitophagy *etc.*).

[0514] In some embodiments, treatment with the mitochondrial-targeted creatine compound will prevent or delay the onset of one or more of the following symptoms: poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation,

memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, brain atrophy, or any other sign or symptom of a mitochondrial disease state disclosed herein.

[0515] In some embodiments, administration of one or more mitochondrial-targeted creatine compounds in subjects with a mitochondrial disease or disorder will cause the levels of one or more energy biomarkers to be similar to that observed in a normal control subject. In certain embodiments, the energy biomarker is selected from the group consisting of lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; total, reduced or oxidized glutathione levels; total, reduced or oxidized cysteine levels; phosphocreatine levels; NADH (NADH+H30) or NADPH (NADPH+H30) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; acetoacetate levels; beta-hydroxy butyrate levels; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; and reactive oxygen species levels. In some embodiments of the method, the lactate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are similar to that observed in a normal control subject. In some embodiments of the method, the pyruvate levels of one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are similar to that observed in a normal control subject. In some embodiments of the method, the total, reduced or oxidized glutathione levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are similar to that observed in a normal control subject. In some embodiments of the method, the total, reduced or oxidized cysteine levels of one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid in a treated subject are similar to that observed in a normal control subject.

[0516] In one aspect, the present technology provides a method for preventing or delaying the onset of Alport Syndrome or symptoms of Alport Syndrome in a subject at risk of having Alport Syndrome. In some embodiments, the subject may exhibit one or more mutations in COL4A3, COL4A4, and COL4A5, which are involved in the production or assembly of type IV collagen fibers.

[0517] Subjects at risk for aberrant levels and/or function of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin compared to a normal control subject or Alport Syndrome can be identified by, *e.g.*, any or a combination of diagnostic or prognostic assays known in the art. In prophylactic applications, pharmaceutical compositions or medicaments of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, are administered to a subject susceptible to, or otherwise at risk of a disease or condition such as *e.g.*, Alport Syndrome, in an amount sufficient to eliminate or reduce the risk, or delay the onset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Administration of a prophylactic mitochondrial-targeted creatine compound can occur prior to the manifestation of symptoms characteristic of the disease or disorder, such that the disease or disorder is prevented or, alternatively, delayed in its progression.

[0518] Subjects at risk for aberrant levels and/or function of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin compared to a normal control subject or Alport Syndrome include, but are not limited to, subjects harboring mutations in COL4A3, COL4A4, and COL4A5, which are involved in the synthesis of type IV collagen fibers.

[0519] In some embodiments, treatment with the mitochondrial-targeted creatine compound will prevent or delay the onset of one or more of the following symptoms: hematuria, proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, GBM ultrastructural abnormalities, nephrotic syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis. In certain embodiments, the urine levels of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin in Alport Syndrome subjects treated with the mitochondrial-targeted creatine compound will resemble those observed in healthy controls. In certain embodiments, Alport Syndrome subjects treated with the mitochondrial-targeted creatine compound will show MMP-9 expression in mesangial cells that is similar to that observed in a normal control subject.

[0520] In one aspect, the present technology provides a method for preventing or delaying the onset of porphyria or symptoms of porphyria in a subject at risk of having porphyria. In some embodiments, the subject may exhibit one or more mutations in genes encoding enzymes required for heme biosynthesis.

[0521] Subjects at risk for aberrant levels and/or function of enzymes involved in heme biosynthesis compared to a normal control subject or porphyria can be identified by, *e.g.*, any or a combination of diagnostic or prognostic assays known in the art. In prophylactic applications, pharmaceutical compositions or medicaments of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, are administered to a subject susceptible to, or otherwise at risk of a disease or condition such as *e.g.*, porphyria, in an amount sufficient to eliminate or reduce the risk, or delay the outset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Administration of a prophylactic mitochondrial-targeted creatine compound can occur prior to the manifestation of symptoms characteristic of the disease or disorder, such that the disease or disorder is prevented or, alternatively, delayed in its progression.

[0522] Subjects at risk for aberrant levels and/or function of enzymes involved in heme biosynthesis compared to a normal control subject, or porphyria include, but are not limited to, subjects harboring mutations in one or more genes encoding enzymes involved in heme biosynthesis.

[0523] In some embodiments, treatment with the mitochondrial-targeted creatine compound will prevent or delay the onset of one or more of the following symptoms: cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, life threatening acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension. In certain embodiments of the method, porphyria subjects treated with the mitochondrial-targeted creatine compound will show normalization of the levels and/or function of one or more enzymes required for heme biosynthesis compared to untreated porphyria subjects. In certain embodiments, the levels and/or function of one or

more enzymes involved in heme biosynthesis in porphyria subjects treated with the mitochondrial-targeted creatine compound will resemble those observed in healthy controls.

[0524] In one aspect, the present technology provides a method for preventing or delaying the onset of vitiligo or symptoms of vitiligo in a subject at risk of having vitiligo. In some embodiments, the subject may exhibit one or more mutations in NLRP1, TYR, HLA class I, HLA class II, HLA class III, PTPN22, XBP1, IL2RA, LPP, RERE, FOXP1, TSLP, CCR6, GZMB, UBASH3A, C1QTNF6, and FOXP3.

[0525] In prophylactic applications, pharmaceutical compositions or medicaments of a mitochondrial-targeted creatine compound or a pharmaceutically acceptable salt thereof, are administered to a subject susceptible to, or otherwise at risk of a disease or condition such as vitiligo, in an amount sufficient to eliminate or reduce the risk, or delay the onset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Administration of a prophylactic mitochondrial-targeted creatine compound can occur prior to the manifestation of symptoms characteristic of the disease or disorder, such that the disease or disorder is prevented or, alternatively, delayed in its progression.

[0526] Subjects at risk for vitiligo include, but are not limited to, subjects harboring mutations in NLRP1, TYR, HLA class I, HLA class II, HLA class III, PTPN22, XBP1, IL2RA, LPP, RERE, FOXP1, TSLP, CCR6, GZMB, UBASH3A, C1QTNF6, and FOXP3.

[0527] In some embodiments, treatment with the mitochondrial-targeted creatine compound will prevent or delay the onset of one or more of the following symptoms: increased photosensitivity, decreased contact sensitivity response to dinitrochlorobenzene, depigmentation of the skin, mucous membranes, retina, or genitals, and premature whitening or graying of hair on the scalp, eyelashes, eyebrows or beard.

[0528] In some embodiments, treatment with the mitochondrial-targeted creatine compound will prevent or delay the onset of one or more of the symptoms of IPF, including but not limited to, an increase in TGF-β1-induced epithelial to mesenchymal transition (EMT), myofibroblast activation, collagen production, lung scarring, and severe progressive fibrosis including fibrotic foci and honeycombing.

[0529] For therapeutic and/or prophylactic applications, a composition comprising a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, is administered to the subject. In some embodiments, the mitochondrial-targeted creatine compound is administered one, two, three, four, or five times per day. In some embodiments, the mitochondrial-targeted creatine compound is administered more than five times per day. Additionally or alternatively, in some embodiments, the mitochondrial-targeted creatine compound is administered every day, every other day, every third day, every fourth day, every fifth day, or every sixth day. In some embodiments, the mitochondrial-targeted creatine compound is administered weekly, bi-weekly, tri-weekly, or monthly. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of one, two, three, four, or five weeks. In some embodiments, the mitochondrial-targeted creatine compound is administered for six weeks or more. In some embodiments, the mitochondrial-targeted creatine compound is administered for twelve weeks or more. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of less than one year. In some embodiments, the mitochondrial-targeted creatine compound is administered for a period of more than one year.

[0530] Additionally or alternatively, in some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for one, two, three, four or five weeks. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for less than 6 weeks. In some embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for 6 weeks or more. In other embodiments of the method, the mitochondrial-targeted creatine compound is administered daily for 12 weeks or more.

Determination of the Biological Effect of the Mitochondrial-Targeted Creatine Compounds of the Present Technology

[0531] In various embodiments, suitable *in vitro* or *in vivo* assays are performed to determine the effect of a specific mitochondrial-targeted creatine compound of the present technology and whether its administration is indicated for treatment. In various embodiments, *in vitro* assays can be performed with representative animal models, to determine if a given mitochondrial-targeted creatine compound-based therapeutic exerts the desired effect in reducing disruption of mitochondrial function, such as disruption of OXPHOS, or alternatively treating a medical disease or condition, such as vitiligo, Alport

Syndrome, porphyria or IPF. Compounds for use in therapy can be tested in suitable animal model systems including, but not limited to rats, mice, chicken, cows, monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo* testing, any of the animal model system known in the art can be used prior to administration to human subjects. In some embodiments, *in vitro* or *in vivo* testing is directed to the biological function of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof.

[0532] The mitochondrial-targeted creatine compounds of the present technology can be tested *in vitro* for efficacy. One such assay is ability of a compound to rescue FRDA fibroblasts stressed by addition of L-buthionine-(S,R)-sulfoximine (BSO), as described in Jauslin *et al.*, *Hum. Mol. Genet.* 11(24):3055 (2002), Jauslin *et al.*, *FASEB J.* 17:1972-4 (2003), and International Patent Application WO 2004/003565. Human dermal fibroblasts from Friedreich's Ataxia patients have been shown to be hypersensitive to inhibition of the *de novo* synthesis of glutathione (GSH) with L-buthionine-(S,R)-sulfoximine (BSO), a specific inhibitor of GSH synthetase (Jauslin *et al.*, *Hum. Mol. Genet.* 11(24):3055 (2002)). This specific BSO-mediated cell death can be prevented by administration of antioxidants or molecules involved in the antioxidant pathway, such as α-tocopherol, short chain quinones, selenium, or small molecule glutathione peroxidase mimetics. However, antioxidants differ in their potency, *i.e.*, the concentration at which they are able to rescue BSO-stressed FRDA fibroblasts. With this assay, EC50 concentrations of the compounds of the present technology can be determined and compared to known reference antioxidants. Similar screens can be applied to fibroblasts derived from patients diagnosed as having, suspected as having, or at risk of having LHON, Huntington's Disease, Parkinson's Disease, CoQ10 deficiencies, *etc.*

[0533] In some embodiments, disruption in oxidative phosphorylation is determined by assays well known in the art. By way of example, but not by way of limitation, a disruption in oxidative phosphorylation is determined by assays that measures levels of coenzyme Q_{10} (COQ10). In some embodiments, disruption in oxidative phosphorylation is determined by assays that measure OXPHOS capacity by the uncoupling ratio. In some embodiments, disruption in oxidative phosphorylation is determined by assays that measure the net routine flux control ratio. In some embodiments, disruption in oxidative phosphorylation is determined by assays that measure leak flux control ratio. In some embodiments, disruption

in oxidative phosphorylation is determined by assays that measure the phosphorylation respiratory control ratio.

[0534] Uncoupling ratio (UCR) is an expression of the respiratory reserve capacity and indicates the OXPHOS capacity of the cells. In some embodiments, UCR is defined as Cr_u / Cr. Cr_u is the maximum rate of oxygen utilization (Oxygen flux) produced when mitochondria are chemically uncoupled using FCCP (Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone). FCCP titration must be performed since the concentration of FCCP required to produce maximum oxygen utilization varies among different cell lines. Once the maximum oxygen utilization is reached, further increases in FCCP inhibit oxygen utilization by oxidative phosphorylation. In some embodiments, Cr represents oxygen utilization by the cells during a normal cellular respiration with excess substrates.

[0535] In some embodiments, the Net Routine Flux Control Ratio (Cr/ Cr_u) is the inverse of the UCR. In some embodiments, this value assesses how close routine respiration operates to the respiratory capacity of oxidative phosphorylation.

[0536] In some embodiments, the Respiratory Control Ratio (RCR) is defined as Cr_u/Cr_o. Cr_u is defined above. Cr_o = Respiration after inhibition of Complex V (ATP synthase) by oligomycin. In some embodiments, this ratio allows assessment of uncoupling and OXPHOS dysfunction.

[0537] In some embodiments, the Leak Flux Control Ratio is determined by Cr_o/Cr_u. In some embodiments, this parameter is the inverse of RCR and represent proton leak with inhibition of ADP phosphorylation by oligomycin.

[0538] In some embodiments, the Phosphorylation Respiratory Control Ratio (RCRp) is defined as (Cr – Cr_o)/ Cr_u (or 1/UCR – 1/RCR). In some embodiments, the RCRp is an index which expresses phosphorylation-related respiration (Cr- Cr_o) as a function of respiratory capacity (Cr_u). In some embodiments, the RCRp remains constant, if partial uncoupling is fully compensated by an increased routine respiration rate and a constant rate of oxidative phosphorylation is maintained. In some embodiments, if the respiratory capacity declines without effect on the rate of oxidative phosphorylation; in some embodiments, the RCRp increases, which indicates that, a higher proportion of the maximum capacity is activated to drive ATP synthesis. In some embodiments, the RCRp declines to zero in either fully uncoupled cells or in cells under complete metabolic arrest.

[0539] Accordingly, in some embodiments, therapeutic and/or prophylactic treatment of subjects having mitochondrial disorder or disease, with a mitochondrial-targeted creatine compound as disclosed herein, or a pharmaceutically acceptable salt thereof, will reduce the disruption in oxidative phosphorylation, thereby ameliorating symptoms of mitochondrial diseases and disorders. Symptoms of mitochondrial diseases or disorders include, but are not limited to, poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, poor growth, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, brain atrophy, or any other sign or symptom of a mitochondrial disease state disclosed herein.

[0540] Animal models of various diseases or conditions described herein (*e.g.*, vitiligo, Alport Syndrome, IPF, porphyria) may be generated using techniques known in the art. Such models may be used to demonstrate the biological effect of mitochondrial-targeted creatine compounds of the present technology, in the prevention and treatment of conditions arising from disruption of a particular gene, and for determining what comprises a therapeutically effective amount of a mitochondrial-targeted creatine compound in a given context.

[0541] In some embodiments, melanocyte degeneration is determined by assays well known in the art. In some embodiments, melanocyte degeneration is determined by assays that measure cytotoxicity after epidermal cells are exposed to 100 or 250μM of 4-tertiary butyl phenol (4-TBP), a common inducer of vitiligo. In some embodiments, melanocyte degeneration is determined by assays that measure the survival rate of epidermal cells that have been exposed to 100 or 250μM of 4-TBP.

[0542] In some embodiments, melanocyte degeneration is determined by assays that measure melanocyte antigen-specific T cell accumulation and cytotoxic activity in autologous skin explants. For a detailed description of the autologous skin explant model, see Van Den Boorn *et al.*, *Journal of Investigative Dermatology*, 129: 2220-2232 (2009).

[0543] In some embodiments, melanocyte degeneration is determined by assays that measure the progressive depigmentation in the pelage of the vitiligo mouse model before and two weeks after plucking dorsal hairs. In some embodiments, melanocyte degeneration is determined by assays that measure the presence of ocular pigmentation in vitiligo mice. In some embodiments, melanocyte degeneration is determined by assays that measure the contact sensitivity of vitiligo mice to dinitrochlorobenzene. For a detailed description of the vitiligo mouse model, see Lerner *et al.*, *Journal of Investigative Dermatology*, 87(3): 299-304 (1986).

[0544] In some embodiments, melanocyte degeneration is determined by assays that measure epidermal depigmentation in an adoptive transfer mouse model of vitiligo. In some embodiments, melanocyte degeneration is determined by assays that measure tyrosinase RNA expression in an adoptive transfer mouse model of vitiligo. For a detailed description of the adoptive transfer mouse model of vitiligo, see Harris *et al.*, *Journal of Investigative Dermatology*, 132: 1869-1876 (2012).

[0545] Accordingly, in some embodiments, therapeutic and/or prophylactic treatment of subjects having vitiligo, with a mitochondrial-targeted creatine compound as disclosed herein, or a pharmaceutically acceptable salt thereof, will reduce melanocyte degeneration, thereby ameliorating symptoms of vitiligo. Symptoms of vitiligo include, but are not limited to, increased photosensitivity, decreased contact sensitivity response to dinitrochlorobenzene, depigmentation of the skin, mucous membranes, retina, or genitals, and premature whitening or graying of hair on the scalp, eyelashes, eyebrows or beard.

[0546] Animal models of Alport Syndrome may be generated using techniques known in the art, including, for example by generating random or targeted mutations in one or more of COL4A3, COL4A4, and COL4A5. For example, murine models of X-linked Alport Syndrome and autosomal recessive Alport Syndrome have been generated by targeted disruption of the mouse Col4a5 gene and mouse Col4a3 gene respectively. See Rheault et al., *J Am Soc Nephrol.* 15(6):1466-74 (2004); Cosgrove *et al.*, *Genes Dev.* 10(23):2981-92 (1996).

[0547] Accordingly, in some embodiments, therapeutic and/or prophylactic treatment of subjects having Alport Syndrome, with a mitochondrial-targeted creatine compound as disclosed herein, or a pharmaceutically acceptable salt thereof, will ameliorate symptoms of

Alport Syndrome. Symptoms of Alport Syndrome include, but are not limited to, hematuria, proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, GBM ultrastructural abnormalities, nephrotic syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis.

[0548] Animals subjected to TGF-β1-adenovirus-induced lung fibrosis or bleomycin-induced lung fibrosis may be used as an *in vivo* model for IPF. MacKinnon *et al.*, *Am. J. Respir. Crit. Care Med.* 185(5):537-46 (2012).

[0549] Accordingly, in some embodiments, therapeutic and/or prophylactic treatment of subjects having IPF, with a mitochondrial-targeted creatine compound as disclosed herein, or a pharmaceutically acceptable salt thereof, will ameliorate symptoms of IPF.

[0550] Animal models of porphyria may be generated using techniques known in the art, including, for example by generating random or targeted mutations in one or more genes encoding enzymes involved in heme biosynthesis. For example, a murine model of familial porphyria cutanea tarda has been generated by targeted disruption of the URO-D gene by homologous recombination.

[0551] Accordingly, in some embodiments, therapeutic and/or prophylactic treatment of subjects having porphyria, with a mitochondrial-targeted creatine compound as disclosed herein, or a pharmaceutically acceptable salt thereof, will ameliorate symptoms of porphyria. Symptoms of porphyria include, but are not limited to, cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension.

Use of Mitochondrial-Targeted Creatine Compounds of the Present Technology for Modulation of Energy Biomarkers

[0552] In addition to monitoring energy biomarkers to assess the status of treatment or suppression of mitochondrial diseases, the mitochondrial-targeted creatine compounds of the present technology can be used in subjects or patients to modulate one or more energy biomarkers. Modulation of energy biomarkers can be done to normalize energy biomarkers in a subject, or to enhance energy biomarkers in a subject.

[0553] Normalization of one or more energy biomarkers is defined as either restoring the level of one or more such energy biomarkers to normal or near-normal levels in a subject whose levels of one or more energy biomarkers show pathological differences from normal levels (*i.e.*, levels in a healthy subject), or to change the levels of one or more energy biomarkers to alleviate pathological symptoms in a subject. Depending on the nature of the energy biomarker, such levels may show measured values either above or below a normal value. For example, a pathological lactate level is typically higher than the lactate level in a normal (*i.e.*, healthy) person, and a decrease in the level may be desirable. A pathological ATP level is typically lower than the ATP level in a normal (*i.e.*, healthy) person, and an increase in the level of ATP may be desirable. Accordingly, normalization of energy biomarkers can involve restoring the level of energy biomarkers to within about at least two standard deviations of normal in a subject, or to within about at least one standard deviation of normal in a subject, to within about at least one-half standard deviation of normal, or to within about at least one-quarter standard deviation of normal.

[0554] When an increase in an energy biomarker level is desired to normalize the one or more such energy biomarker, the level of the energy biomarker can be increased to within about at least two standard deviations of normal in a subject, increased to within about at least one standard deviation of normal in a subject, increased to within about at least one-half standard deviation of normal, or increased to within about at least one-quarter standard deviation of normal, by administration of one or more compounds according to the present technology. Alternatively, the level of one or more of the energy biomarkers can be increased by about at least 10% above the subject's level of the respective one or more energy biomarkers before administration; by about at least 20% above the subject's level of the respective one or more energy biomarkers before administration, by about at least 30% above the subject's level of the respective one or more energy biomarkers before administration, by

about at least 40% above the subject's level of the respective one or more energy biomarkers before administration, by about at least 50% above the subject's level of the respective one or more energy biomarkers before administration, by about at least 75% above the subject's level of the respective one or more energy biomarkers before administration, or by about at least 100% above the subject's level of the respective one or more energy biomarkers before administration.

[0555] When a decrease in a level of one or more energy biomarkers is desired to normalize the one or more energy biomarkers, the level of the one or more energy biomarkers can be decreased to a level within about at least two standard deviations of normal in a subject, decreased to within about at least one standard deviation of normal in a subject, decreased to within about at least one-half standard deviation of normal, or decreased to within about at least one-quarter standard deviation of normal, by administration of one or more compounds according to the present technology. Alternatively, the level of the one or more energy biomarkers can be decreased by about at least 10% below the subject's level of the respective one or more energy biomarkers before administration, by about at least 20% below the subject's level of the respective one or more energy biomarkers before administration, by about at least 30% below the subject's level of the respective one or more energy biomarkers before administration, by about at least 40% below the subject's level of the respective one or more energy biomarkers before administration, by about at least 50% below the subject's level of the respective one or more energy biomarkers before administration, by about at least 75% below the subject's level of the respective one or more energy biomarkers before administration, or by about at least 90% below the subject's level of the respective one or more energy biomarkers before administration.

[0556] Enhancement of the level of one or more energy biomarkers is defined as changing the extant levels of one or more energy biomarkers in a subject to a level which provides beneficial or desired effects for the subject. For example, a person undergoing strenuous effort or prolonged vigorous physical activity, such as mountain climbing, could benefit from increased ATP levels or decreased lactate levels. As described above, normalization of energy biomarkers may not achieve the optimum state for a subject with a mitochondrial disease, and such subjects can also benefit from enhancement of energy biomarkers. Examples of subjects who could benefit from enhanced levels of one or more energy biomarkers include, but are not limited to, subjects undergoing strenuous or prolonged

physical activity, subjects with chronic energy problems, or subjects with chronic respiratory problems. Such subjects include, but are not limited to, pregnant females, particularly pregnant females in labor; neonates, particularly premature neonates; subjects exposed to extreme environments, such as hot environments (temperatures routinely exceeding about 85-86 degrees Fahrenheit or about 30 degrees Celsius for about 4 hours daily or more), cold environments (temperatures routinely below about 32 degrees Fahrenheit or about 0 degrees Celsius for about 4 hours daily or more), or environments with lower-than-average oxygen content, higher-than-average carbon dioxide content, or higher-than-average levels of air pollution (airline travelers, flight attendants, subjects at elevated altitudes, subjects living in cities with lower-than average air quality, subjects working in enclosed environments where air quality is degraded); subjects with lung diseases or lower-than-average lung capacity, such as tubercular patients, lung cancer patients, emphysema patients, and cystic fibrosis patients; subjects recovering from surgery or illness; elderly subjects, including elderly subjects experiencing decreased energy; subjects suffering from chronic fatigue, including chronic fatigue syndrome; subjects undergoing acute trauma; subjects in shock; subjects requiring acute oxygen administration; subjects requiring chronic oxygen administration; or other subjects with acute, chronic, or ongoing energy demands who can benefit from enhancement of energy biomarkers.

[0557] In another embodiment of the present technology, including any of the foregoing embodiments, the mitochondrial-targeted creatine compounds described herein are administered to subjects suffering from a mitochondrial disorder to modulate one or more of various energy biomarkers, including, but not limited to, lactic acid (lactate) levels (in one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid); pyruvic acid (pyruvate) levels (in one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid); lactate/pyruvate ratios (in one or more of whole blood, plasma, cerebrospinal fluid, or cerebral ventricular fluid); total, reduced or oxidized glutathione levels, or reduced/oxidized glutathione ratios (in one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid); total, reduced or oxidized cysteine levels, or reduced/oxidized cysteine ratios (in one or more of whole blood, plasma, lymphocytes, cerebrospinal fluid, or cerebral ventricular fluid); phosphocreatine levels; NADH (NADH+H^{30}) or NADPH (NADPH+H^{30}) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; oxidized

cytochrome C/reduced cytochrome C ratio; acetoacetate levels; beta-hydroxy butyrate levels; acetoacetate/ beta-hydroxy butyrate ratio; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; levels of reactive oxygen species; oxygen consumption (VO2), carbon dioxide output (VCO2), respiratory quotient (VCO2/VO2), and to modulate exercise intolerance (or conversely, modulate exercise tolerance) and to modulate anaerobic threshold. Energy biomarkers can be measured in whole blood, plasma, cerebrospinal fluid, cerebroventricular fluid, arterial blood, venous blood, or any other body fluid, body gas, or other biological sample useful for such measurement. In one embodiment, the levels are modulated to a value within about 2 standard deviations of the value in a healthy subject. In another embodiment, the levels are modulated to a value within about 1 standard deviation of the value in a healthy subject. In another embodiment, the levels in a subject are changed by at least about 10% above or below the level in the subject prior to modulation. In another embodiment, the levels are changed by at least about 20% above or below the level in the subject prior to modulation. In another embodiment, the levels are changed by at least about 30% above or below the level in the subject prior to modulation. In another embodiment, the levels are changed by at least about 40% above or below the level in the subject prior to modulation. In another embodiment, the levels are changed by at least about 50% above or below the level in the subject prior to modulation. In another embodiment, the levels are changed by at least about 75% above or below the level in the subject prior to modulation. In another embodiment, the levels are changed by at least about 100% above or at least about 90% below the level in the subject prior to modulation.

[0558] Several metabolic biomarkers have already been used to evaluate efficacy of CoQ10, and these metabolic biomarkers can be monitored as energy biomarkers for use in the methods of the present technology. Pyruvate, a product of the anaerobic metabolism of glucose, is removed by reduction to lactic acid in an anaerobic setting or by oxidative metabolism, which is dependent on a functional mitochondrial respiratory chain. Dysfunction of the respiratory chain may lead to inadequate removal of lactate and pyruvate from the circulation and elevated lactate/pyruvate ratios are observed in mitochondrial cytopathies (see Scriver C R, *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., New York: McGraw-Hill, Health Professions Division, 1995; and Munnich *et al.*, *J. Inherit. Metab. Dis.* 15(4):448-55 (1992)). Blood lactate/pyruvate ratio (Chariot *et al.*, *Arch. Pathol. Lab. Med.* 118(7):695-7 (1994)) is, therefore, widely used as a noninvasive test for detection of mitochondrial cytopathies (see again Scriver C R, *The Metabolic and Molecular*

Bases of Inherited Disease, 7th ed., New York: McGraw-Hill, Health Professions Division, 1995; and Munnich *et al.*, *J. Inherit. Metab. Dis.* 15(4):448-55 (1992)) and toxic mitochondrial myopathies (Chariot *et al.*, *Arthritis Rheum.* 37(4):583-6 (1994)). Changes in the redox state of liver mitochondria can be investigated by measuring the arterial ketone body ratio (acetoacetate/3-hydroxybutyrate: AKBR) (Ueda *et al.*, *J. Cardiol.* 29(2):95-102 (1997)). Urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) often has been used as a biomarker to assess the extent of repair of ROS-induced DNA damage in both clinical and occupational settings (Erhola *et al.*, *FEBS Lett.* 409(2):287-91 (1997); Honda *et al.*, *Leuk. Res.* 24(6):461-8 (2000); Pilger *et al.*, *Free Radic. Res.* 35(3):273-80 (2001); Kim *et al.* *Environ Health Perspect* 112(6):666-71 (2004)).

[0559] Magnetic resonance spectroscopy (MRS) has been useful in the diagnoses of mitochondrial cytopathy by demonstrating elevations in cerebrospinal fluid (CSF) and cortical white matter lactate using proton MRS (1H-MRS) (Kaufmann *et al.*, *Neurology* 62(8):1297-302 (2004)). Phosphorous MRS (31P-MRS) has been used to demonstrate low levels of cortical phosphocreatine (PCr) (Matthews *et al.*, *Ann. Neurol.* 29(4):435-8 (1991)), and a delay in PCr recovery kinetics following exercise in skeletal muscle (Matthews *et al.*, *Ann. Neurol.* 29(4):435-8 (1991); Barbiroli *et al.*, *J. Neurol.* 242(7):472-7 (1995); Fabrizi *et al.*, *J. Neurol. Sci.* 137(1):20-7 (1996)). A low skeletal muscle PCr has also been confirmed in patients with mitochondrial cytopathy by direct biochemical measurements.

[0560] Exercise testing is particularly helpful as an evaluation and screening tool in mitochondrial myopathies. One of the hallmark characteristics of mitochondrial myopathies is a reduction in maximal whole body oxygen consumption (VO2max) (Taivassalo *et al.*, *Brain* 126(Pt 2):413-23 (2003)). Given that VO2max is determined by cardiac output (Qc) and peripheral oxygen extraction (arterial-venous total oxygen content) difference, some mitochondrial cytopathies affect cardiac function where delivery can be altered; however, most mitochondrial myopathies show a characteristic deficit in peripheral oxygen extraction (A-VO2 difference) and an enhanced oxygen delivery (hyperkinetic circulation) (Taivassalo *et al.*, *Brain* 126(Pt 2):413-23 (2003)). This can be demonstrated by a lack of exercise induced deoxygenation of venous blood with direct AV balance measurements (Taivassalo *et al.*, *Ann. Neurol.* 51(1):38-44 (2002)) and non-invasively by near infrared spectroscopy (Lynch *et al.*, *Muscle Nerve* 25(5):664-73 (2002); van Beekvelt *et al.*, *Ann. Neurol.* 46(4):667-70 (1999)).

[0561] Several of these energy biomarkers are discussed in more detail as follows. It should be emphasized that, while certain energy biomarkers are discussed and enumerated herein, the present technology is not limited to modulation, normalization or enhancement of only these enumerated energy biomarkers.

[0562] Lactic acid (lactate) levels: Mitochondrial dysfunction typically results in abnormal levels of lactic acid, as pyruvate levels increase and pyruvate is converted to lactate to maintain capacity for glycolysis. Mitochondrial dysfunction can also result in abnormal levels of $NADH+H^{30}$, $NADPH+H^{30}$, NAD, or NADP, as the reduced nicotinamide adenine dinucleotides are not efficiently processed by the respiratory chain. Lactate levels can be measured by taking samples of appropriate bodily fluids such as whole blood, plasma, or cerebrospinal fluid. Using magnetic resonance, lactate levels can be measured in virtually any volume of the body desired, such as the brain.

[0563] Measurement of cerebral lactic acidosis using magnetic resonance in patients is described in Kaufmann *et al.*, *Neurology* 62(8): 1297 (2004). Whole blood, plasma, and cerebrospinal fluid lactate levels can be measured by commercially available equipment such as the YSI 2300 STAT Plus Glucose & Lactate Analyzer (YSI Life Sciences, Ohio).

[0564] NAD, NADP, NADH and NADPH levels: Measurement of NAD, NADP, NADH ($NADH+H^{30}$) or NADPH ($NADPH+H^{30}$) can be measured by a variety of fluorescent, enzymatic, or electrochemical techniques, *e.g.*, the electrochemical assay described in US 2005/0067303.

[0565] Oxygen consumption (vO_2 or VO2), carbon dioxide output (vCO_2 or VCO2), and respiratory quotient (VCO2/VO2): vO_2 is usually measured either while resting (resting vO_2) or at maximal exercise intensity (vO_2 max). Optimally, both values will be measured. However, for severely disabled patients, measurement of vO_2 max may be impractical. Measurement of both forms of vO_2 is readily accomplished using standard equipment from a variety of vendors, *e.g.*, Korr Medical Technologies, Inc. (Salt Lake City, Utah). VCO2 can also be readily measured, and the ratio of VCO2 to VO2 under the same conditions (VCO2/VO2, either resting or at maximal exercise intensity) provides the respiratory quotient (RQ).

[0566] Oxidized Cytochrome C, reduced Cytochrome C, and ratio of oxidized Cytochrome C to reduced Cytochrome C: Cytochrome C parameters, such as oxidized cytochrome C

levels (Cyt C_{ox}), reduced cytochrome C levels (Cyt C_{red}), and the ratio of oxidized cytochrome C/reduced cytochrome C ratio (Cyt C_{ox})/(Cyt C_{red}), can be measured by *in vivo* near infrared spectroscopy. See, *e.g.*, Rolfe, P., "*In vivo* near-infrared spectroscopy," *Annu. Rev. Biomed. Eng.* 2:715-54 (2000) and Strangman *et al.*, "Non-invasive neuroimaging using near-infrared light" *Biol. Psychiatry* 52:679-93 (2002).

[0567] Exercise tolerance/Exercise intolerance: Exercise intolerance is defined as "the reduced ability to perform activities that involve dynamic movement of large skeletal muscles because of symptoms of dyspnea or fatigue" (Pina *et al.*, *Circulation* 107:1210 (2003)). Exercise intolerance is often accompanied by myoglobinuria, due to breakdown of muscle tissue and subsequent excretion of muscle myoglobin in the urine. Various measures of exercise intolerance can be used, such as time spent walking or running on a treadmill before exhaustion, time spent on an exercise bicycle (stationary bicycle) before exhaustion, and the like. Treatment with the compositions or methods of the present technology can result in about a 10% or greater improvement in exercise tolerance (for example, about a 10% or greater increase in time to exhaustion, *e.g.*, from 10 minutes to 11 minutes), about a 20% or greater improvement in exercise tolerance, about a 30% or greater improvement in exercise tolerance, about a 40% or greater improvement in exercise tolerance, about a 50% or greater improvement in exercise tolerance, about a 75% or greater improvement in exercise tolerance, or about a 100% or greater improvement in exercise tolerance. While exercise tolerance is not, strictly speaking, an energy biomarker, for the purposes of the present technology, modulation, normalization, or enhancement of energy biomarkers includes modulation, normalization, or enhancement of exercise tolerance.

[0568] Similarly, tests for normal and abnormal values of pyruvic acid (pyruvate) levels, lactate/pyruvate ratio, ATP levels, anaerobic threshold, reduced coenzyme Q (CoQ^{red}) levels, oxidized coenzyme Q (CoQ^{ox}) levels, total coenzyme Q (CoQ^{tot}) levels, oxidized cytochrome C levels, reduced cytochrome C levels, oxidized cytochrome C/reduced cytochrome C ratio, acetoacetate levels, β-hydroxy butyrate levels, acetoacetate/β-hydroxy butyrate ratio, 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, and levels of reactive oxygen species are known in the art and can be used to evaluate efficacy of the compositions and methods of the present technology. (For the purposes of the present technology, modulation, normalization, or enhancement of energy biomarkers includes modulation, normalization, or enhancement of anaerobic threshold.)

[0569] Neuroimaging is indicated in individuals with suspected CNS disease. CT may show basal ganglia calcification and/or diffuse atrophy. MRI may show focal atrophy of the cortex or cerebellum, or high signal change on T2-weighted images, particularly in the occipital cortex. There may also be evidence of a generalized leukoencephalopathy. Cerebellar atrophy is a prominent feature in children.

[0570] Electroencephalography (EEG) is indicated in individuals with suspected encephalopathy or seizures. Encephalopathy may be associated with generalized slow wave activity on the EEG. Generalized or focal spike and wave discharges may be seen in individuals with seizures.

[0571] Peripheral neurophysiologic studies are indicated in individuals with limb weakness, sensory symptoms, or areflexia. Electromyography (EMG) is often normal but may show myopathic features. Nerve conduction velocity (NCV) may be normal or may show a predominantly axonal sensorimotor polyneuropathy.

[0572] Magnetic resonance spectroscopy (MRS) and exercise testing (with measurement of blood concentration of lactate) may be used to detect evidence of abnormal mitochondrial function non-invasively.

[0573] *Glucose*. An elevated concentration of fasting blood glucose may indicate diabetes mellitus.

[0574] *Cardiac*. Both electrocardiography and echocardiography may indicate cardiac involvement (cardiomyopathy or atrioventricular conduction defects).

[0575] Treatment of a subject afflicted by a mitochondrial disease in accordance with the methods of the present technology may result in the inducement of a reduction or alleviation of symptoms in the subject, *e.g.*, to halt the further progression of the disorder. Partial or complete suppression of the mitochondrial disease can result in a lessening of the severity of one or more of the symptoms that the subject would otherwise experience.

[0576] Any one, or any combination of, the energy biomarkers described herein (*e.g.*, Figure 1) provide conveniently measurable benchmarks by which to gauge the effectiveness of treatment or suppressive therapy. Additionally, other energy biomarkers are known to

those skilled in the art and can be monitored to evaluate the efficacy of treatment or suppressive therapy.

Modes of Administration and Effective Dosages

[0577] Any method known to those in the art for contacting a cell, organ or tissue with a mitochondrial-targeted creatine compound of the present technology, or a pharmaceutically acceptable salt thereof, may be employed. Suitable methods include *in vitro, ex vivo, or in vivo* methods. *In vivo* methods typically include the administration of a mitochondrial-targeted creatine compound, such as those described above, to a mammal, suitably a human. When used *in vivo* for therapy, mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, are administered to the subject in effective amounts (*i.e.,* amounts that have desired therapeutic effect). The dose and dosage regimen will depend upon the degree of the infection in the subject, the characteristics of the particular mitochondrial-targeted creatine compound used, *e.g.*, its therapeutic index, the subject, and the subject's history.

[0578] The effective amount may be determined during pre-clinical trials and clinical trials by methods familiar to physicians and clinicians. An effective amount of a mitochondrial-targeted creatine compound useful in the methods may be administered to a mammal in need thereof by any of a number of well-known methods for administering pharmaceutical compounds. The mitochondrial-targeted creatine compound may be administered systemically or locally.

[0579] The mitochondrial-targeted creatine compound may be formulated as a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" means a salt prepared from a base or an acid which is acceptable for administration to a patient, such as a mammal (*e.g.,* salts having acceptable mammalian safety for a given dosage regime). However, it is understood that the salts are not required to be pharmaceutically acceptable salts, such as salts of intermediate compounds that are not intended for administration to a patient. Pharmaceutically acceptable salts can be derived from pharmaceutically acceptable inorganic or organic bases and from pharmaceutically acceptable inorganic or organic acids. In addition, when a mitochondrial-targeted creatine compound contains both a basic moiety, such as an amine, pyridine or imidazole, and an acidic moiety such as a carboxylic acid or tetrazole, zwitterions may be formed and are included within the term "salt" as used herein. Salts derived from pharmaceutically acceptable inorganic bases include ammonium, calcium,

copper, ferric, ferrous, lithium, magnesium, manganic, manganous, potassium, sodium, and zinc salts, and the like. Salts derived from pharmaceutically acceptable organic bases include salts of primary, secondary and tertiary amines, including substituted amines, cyclic amines, naturally-occurring amines and the like, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperadine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like. Salts derived from pharmaceutically acceptable inorganic acids include salts of boric, carbonic, hydrohalic (hydrobromic, hydrochloric, hydrofluoric or hydroiodic), nitric, phosphoric, sulfamic and sulfuric acids. Salts derived from pharmaceutically acceptable organic acids include salts of aliphatic hydroxyl acids (*e.g.*, citric, gluconic, glycolic, lactic, lactobionic, malic, and tartaric acids), aliphatic monocarboxylic acids (*e.g.*, acetic, butyric, formic, propionic and trifluoroacetic acids), amino acids (*e.g.*, aspartic and glutamic acids), aromatic carboxylic acids (*e.g.*, benzoic, p-chlorobenzoic, diphenylacetic, gentisic, hippuric, and triphenylacetic acids), aromatic hydroxyl acids (*e.g.*, o-hydroxybenzoic, p-hydroxybenzoic, 1-hydroxynaphthalene-2-carboxylic and 3-hydroxynaphthalene-2-carboxylic acids), ascorbic, dicarboxylic acids (*e.g.*, fumaric, maleic, oxalic and succinic acids), glucuronic, mandelic, mucic, nicotinic, orotic, pamoic, pantothenic, sulfonic acids (*e.g.*, benzenesulfonic, camphosulfonic, edisylic, ethanesulfonic, isethionic, methanesulfonic, naphthalenesulfonic, naphthalene-1,5-disulfonic, naphthalene-2,6-disulfonic and p-toluenesulfonic acids), xinafoic acid, and the like.

[0580] The mitochondrial-targeted creatine compounds described herein, or pharmaceutically acceptable salts thereof, can be incorporated into pharmaceutical compositions for administration, singly or in combination, to a subject for the treatment or prevention of a disorder described herein. Such compositions typically include the active agent and a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" includes saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

[0581] Pharmaceutical compositions are typically formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral (*e.g.*, intravenous, intradermal, intraperitoneal or subcutaneous), oral, inhalation, intrathecal, transdermal (topical), intraocular, iontophoretic, and transmucosal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. For convenience of the patient or treating physician, the dosing formulation can be provided in a kit containing all necessary equipment (*e.g.*, vials of drug, vials of diluent, syringes and needles) for a treatment course (*e.g.*, 7 days of treatment).

[0582] Pharmaceutical compositions suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, a composition for parenteral administration must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

[0583] Compositions containing a mitochondrial-targeted creatine compound can include a carrier, which can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens,

chlorobutanol, phenol, ascorbic acid, thiomerasol, and the like. Glutathione and other antioxidants can be included to prevent oxidation. In some embodiments, compositions containing a mitochondrial-targeted creatine compound include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate or gelatin.

[0584] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, typical methods of preparation include vacuum drying and freeze drying, which can yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0585] Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0586] For administration by inhalation, the compounds can be delivered in the form of an aerosol spray from a pressurized container or dispenser, which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Such methods include those described in U.S. Pat. No. 6,468,798.

[0587] Systemic administration of a therapeutic compound as described herein can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. In one embodiment, transdermal administration may be performed by iontophoresis.

[0588] A therapeutic mitochondrial-targeted creatine compound can be formulated in a carrier system. The carrier can be a colloidal system. The colloidal system can be a liposome, a phospholipid bilayer vehicle. In one embodiment, the therapeutic mitochondrial-targeted creatine compound is encapsulated in a liposome while maintaining its structural integrity. As one skilled in the art would appreciate, there are a variety of methods to prepare liposomes. (*See* Lichtenberg, *et al.*, *Methods Biochem. Anal.*, 33:337-462 (1988); Amselem, *et al.*, *Liposome Technology*, CRC Press (1993)). Liposomal formulations can delay clearance and increase cellular uptake (*See* Reddy, *Ann. Pharmacother.*, 34(7-8):915-923 (2000)). An active agent can also be loaded into a particle prepared from pharmaceutically acceptable ingredients including, but not limited to, soluble, insoluble, permeable, impermeable, biodegradable or gastroretentive polymers or liposomes. Such particles include, but are not limited to, nanoparticles, biodegradable nanoparticles, microparticles, biodegradable microparticles, nanospheres, biodegradable nanospheres, microspheres, biodegradable microspheres, capsules, emulsions, liposomes, micelles and viral vector systems.

[0589] The carrier can also be a polymer, *e.g.*, a biodegradable, biocompatible polymer matrix. In one embodiment, the therapeutic mitochondrial-targeted creatine compound can be embedded in the polymer matrix, while maintaining protein integrity. The polymer may be natural, such as polysaccharides, or synthetic, such as poly α-hydroxy acids. Examples include carriers made of, *e.g.*, collagen, fibronectin, elastin, cellulose acetate, cellulose nitrate, polysaccharide, fibrin, gelatin, and combinations thereof. In one embodiment, the polymer is poly-lactic acid (PLA) or copoly lactic/glycolic acid (PGLA). The polymeric matrices can be prepared and isolated in a variety of forms and sizes, including microspheres

and nanospheres. Polymer formulations can lead to prolonged duration of therapeutic effect. (*See* Reddy, *Ann. Pharmacother.*, 34(7-8):915-923 (2000)). A polymer formulation for human growth hormone (hGH) has been used in clinical trials. (*See* Kozarich and Rich, *Chemical Biology*, 2:548-552 (1998)).

[0590] Examples of polymer microsphere sustained release formulations are described in PCT publication WO 99/15154 (Tracy, *et al.*), U.S. Pat. Nos. 5,674,534 and 5,716,644 (both to Zale, *et al.*), PCT publication WO 96/40073 (Zale, *et al.*), and PCT publication WO 00/38651 (Shah, *et al.*). U.S. Pat. Nos. 5,674,534 and 5,716,644 and PCT publication WO 96/40073 describe a polymeric matrix containing particles of erythropoietin that are stabilized against aggregation with a salt.

[0591] In some embodiments, the therapeutic compounds are prepared with carriers that will protect the therapeutic compounds against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using known techniques. The materials can also be obtained commercially, *e.g.*, from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to specific cells with monoclonal antibodies to cell-specific antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

[0592] The therapeutic compounds can also be formulated to enhance intracellular delivery. For example, liposomal delivery systems are known in the art, see, *e.g.*, Chonn and Cullis, "Recent Advances in Liposome Drug Delivery Systems," *Current Opinion in Biotechnology* 6:698-708 (1995); Weiner, "Liposomes for Protein Delivery: Selecting Manufacture and Development Processes," *Immunomethods*, 4(3):201-9 (1994); and Gregoriadis, "Engineering Liposomes for Drug Delivery: Progress and Problems," *Trends Biotechnol.*, 13(12):527-37 (1995). Mizuguchi, *et al.*, *Cancer Lett.*, 100:63-69 (1996), describes the use of fusogenic liposomes to deliver a protein to cells both *in vivo* and *in vitro*.

[0593] Dosage, toxicity and therapeutic efficacy of the therapeutic agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals,

e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0594] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. In some embodiments, the dosage of such compounds lies within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to determine useful doses in humans accurately. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0595] Typically, an effective amount of a mitochondrial-targeted creatine compound, sufficient for achieving a therapeutic or prophylactic effect, ranges from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Suitably, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 100 mg per kilogram body weight per day. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight every day, every two days or every three days or within the range of 1-10 mg/kg every week, every two weeks or every three weeks. In one embodiment, a single dosage of a mitochondrial-targeted creatine compound ranges from 0.001-10,000 micrograms per kg body weight. In one embodiment, mitochondrial-targeted creatine compound concentrations in a carrier range from 0.2 to 2000 micrograms per delivered milliliter. An exemplary treatment regime entails administration once per day or once a week. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and in certain

embodiments, until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[0596] In some embodiments, a therapeutically effective amount of a mitochondrial-targeted creatine compound may be defined as a concentration of the mitochondrial-targeted creatine compound at the target tissue of 10^{-12} to 10^{-6} molar, *e.g.*, approximately 10^{-7} molar. This concentration may be delivered by systemic doses of 0.001 to 100 mg/kg or equivalent dose by body surface area. In some embodiments, the schedule of doses would be optimized to maintain the therapeutic concentration at the target tissue, by single daily or weekly administration, but also including continuous administration (*e.g.*, parenteral infusion or transdermal application).

[0597] The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to, the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the therapeutic compositions described herein can include a single treatment or a series of treatments.

[0598] The mammal treated in accordance present methods can be any mammal, including, for example, farm animals, such as sheep, pigs, cows, and horses; pet animals, such as dogs and cats; laboratory animals, such as rats, mice and rabbits. In one embodiment, the mammal is a human.

Combination Therapy with a Mitochondrial-Targeted Creatine Compound and Other Therapeutic Agents

[0599] In one embodiment, an additional therapeutic agent is administered to a subject in combination with a mitochondrial-targeted creatine compound of the present technology, such that a synergistic therapeutic effect is produced. In one embodiment, the administration of the mitochondrial-targeted creatine compound in combination with an additional therapeutic agent "primes" the tissue, so that it is more responsive to the therapeutic effects of one or more therapeutic agents.

[0600] In any case, the multiple therapeutic agents may be administered in any order. In some embodiments, the subject is administered multiple therapeutic agents simultaneously,

separately, or sequentially. If simultaneously, the multiple therapeutic agents may be provided in a single, unified form, or in multiple forms (by way of example only, either as a single pill or as two separate pills). One of the therapeutic agents may be given in multiple doses, or both may be given as multiple doses. If not simultaneous, the timing between the multiple doses may vary from more than zero weeks to less than four weeks. In some embodiments, the mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, is administered prior to or subsequent to additional therapeutic agent. In some embodiments, the subject is administered the multiple therapeutic agents before the signs, symptoms or complications of a disease or condition are evident. In addition, the combination methods, compositions and formulations are not to be limited to the use of only two agents.

[0601] By way of example, but not by way of limitation, the treatment for mitochondrial diseases or disorders typically involves taking vitamins and cofactors. In addition, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents, by way of non-limiting example, may also be administered.

[0602] In one embodiment, mitochondrial-targeted creatine compounds of the present technology are combined with one or more cofactors, vitamins, iron chelators, antioxidants, frataxin level modifiers, ACE inhibitors and β-blockers. By way of example, but not by way of limitation, such compounds may include one or more of CoQ10, Levocarnitine, riboflavin, acetyl-L-carnitine, thiamine, nicotinamide, vitamin E, vitamin C, lipoic acid, selenium, β-carotene, biotin, folic acid, calcium, magnesium, phosphorous, succinate, selenium, creatine, uridine, citratesm prednisone, vitamin K, deferoxamine, deferiprone, idebenone, erythropoietin, 17β-estradiol, methylene blue, and histone deacetylase inhibitors such as BML-210 and compound 106.

[0603] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of various anti-oxidant compounds including, but not limited to, *e.g.*, parenteral or oral administration of compositions comprising glycyrrhizin, schisandra, ascorbic acid, L-glutathione, silymarin, lipoic acid, and D-alpha-tocopherol (*see* U.S. Pat. No. 7,078,064, incorporated expressly by reference for all purposes).

[0604] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of various anti-oxidant compounds including, but not limited to, *e.g.,* parenteral or oral administration of compositions comprising a water soluble Vitamin E preparation, mixed carotenoids, or selenium (*see* U.S. Pat. No. 6,596,762, incorporated expressly by reference for all purposes).

[0605] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of parenteral or oral administration of lecithin or vitamin B complex (*see* U.S. Pat. Nos. 7,018,652; 6.180,139, incorporated expressly by reference for all purposes).

[0606] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of bile salt preparations including, but not limited to, *e.g.,* ursodeoxycholic acid, chenodeoxycholic acid of other naturally occurring or synthetic bile acids or bile acid salts (s*ee* U.S. Pat. No. 6.297,229, incorporated expressly by reference for all purposes).

[0607] In some embodiments, mitochondrial-targeted creatine compounds of the present technology, can be used in combination with a therapeutically effective amount of a PPAR (peroxisome proliferator-activated receptor) activity regulators (*see* U.S. Pat. No. 7,994,353, incorporated expressly by reference for all purposes).

[0608] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a benzothiazepine or benzothiepine compound represented by the following formula having a thioamide bond and a quaternary ammonium substituent (*see* U.S. Pat. No. 7,973.030, incorporated expressly by reference for all purposes).

[0609] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a mineralocorticoid receptor antagonist, for example, but not limited to, spironolactone and eplerenone.

[0610] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a beta-

adrenergic antagonist (beta-blocker), for example, but not limited to, metoprolol, bisoprolol, carvedilol, atenolol, and nebivolol.

[0611] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a axacyclopentane derivative that inhibits stearoyl-coenzyme alpha delta-9 desaturase (*see* U.S. Pat. No. 7,754,745, incorporated expressly by reference for all purposes).

[0612] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a acylamide compound having secretagogue or inducer activity of adiponectin (*see* U.S. Pat. No. 7,732,637, incorporated expressly herein by reference for all purposes).

[0613] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of quaternary ammonium compounds (*see* U.S. Pat. No. 7,312,208, incorporated expressly by reference for all purposes).

[0614] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of an isoflavone compound (s*ee* U.S. Pat. No. 6,592,910, incorporated expressly by reference for all purposes).

[0615] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a macrolide antibiotic (s*ee* U.S. Pat. No. 5,760,010, incorporated expressly by reference for all purposes).

[0616] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of carnitine.

[0617] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a statin, for example, but not limited to, HMG-CoA reductase inhibitors such as atorvastatin and simvastatin.

[0618] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of an N-acetyl cysteine.

[0619] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of another galectin inhibitor that may inhibit a single galectin protein or multiple galectin proteins, including, but not limited to, *e.g.*, small organic inhibitors of galectin, monoclonal antibodies, RNA inhibitors, or protein inhibitors.

[0620] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a monoclonal antibody to inhibit lysyl oxidase or monoclonal antibody that binds to connective tissue growth factor.

[0621] In another embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of pentraxin proteins, including, but not limited to, *e.g.*, recombinant pentraxin-2.

[0622] In another embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of an angiotensin receptor blocker (ARB) or an angiotensin-converting enzyme (ACE) inhibitor.

[0623] In another embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a cGMP activating compound.

[0624] In another embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a calcium channel blocker, for example, but not limited to, verapamil.

[0625] In another embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a phosphodiesterase 5 inhibitor, for example, but not limited to, sildenafil, tadalafil, or vardenafil.

[0626] In some embodiments mitochondrial-targeted creatine compounds of the present technology can be used in combination with a therapeutically effective amount of a diuretic.

[0627] In some embodiments, mitochondrial-targeted creatine compounds of the present technology can be used in combination with one or more additional agents selected from the group consisting of diuretics, ACE inhibitors, digoxin (also called digitalis), calcium channel blockers, and beta-blockers. In some embodiments, thiazide diuretics, such as hydrochlorothiazide at 25-50 mg/day or chlorothiazide at 250-500 mg/day, can be used. However, supplemental potassium chloride may be needed, since chronic diuresis causes hypokalemis alkalosis. Typical doses of ACE inhibitors include captopril at 25-50 mg/day and quinapril at 10 mg/day.

[0628] In one embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with an adrenergic beta-2 agonist. An "adrenergic beta-2 agonist" refers to adrenergic beta-2 agonists and analogues and derivatives thereof, including, for example, natural or synthetic functional variants which have adrenergic beta-2 agonist biological activity, as well as fragments of an adrenergic beta-2 agonist having adrenergic beta-2 agonist biological activity. The term "adrenergic beta-2 agonist biological activity" refers to activity that mimics the effects of adrenaline and noradrenaline in a subject and which improves myocardial contractility in a patient having heart failure. Commonly known adrenergic beta-2 agonists include, but are not limited to, *e.g.*, clenbuterol, albuterol, formeoterol, levalbuterol, metaproterenol, pirbuterol, salmeterol, and terbutaline.

[0629] In one embodiment, mitochondrial-targeted creatine compounds of the present technology can be used in combination with an adrenergic beta-1 antagonist. Adrenergic beta-1 antagonists and adrenergic beta-1 blockers refer to adrenergic beta-1 antagonists and analogues and derivatives thereof, including, for example, natural or synthetic functional variants which have adrenergic beta-1 antagonist biological activity, as well as fragments of an adrenergic beta-1 antagonist having adrenergic beta-1 antagonist biological activity. Adrenergic beta-1 antagonist biological activity refers to activity that blocks the effects of adrenaline on beta receptors. Commonly known adrenergic beta-1 antagonists include, but are not limited to, acebutolol, atenolol, betaxolol, bisoprolol, esmolol, and metoprolol.

[0630] Clenbuterol, for example, is available under numerous brand names including Spiropent® (Boehinger Ingelheim), Broncodil® (Von Boch I), Broncoterol® (Quimedical

PT), Cesbron® (Fidelis PT), and Clenbuter® (Biomedica Foscama). Similarly, methods of preparing adrenergic beta-1 antagonists such as metoprolol and their analogues and derivatives are well-known in the art. Metoprolol, in particular, is commercially available under the brand names Lopressor® (metoprolol tartate) manufactured by Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, N.J. 07936-1080. Generic versions of Lopressor® are also available from Mylan Laboratories Inc., 1500 Corporate Drive, Suite 400, Canonsburg, Pa. 15317; and Watson Pharmaceuticals, Inc., 360 Mt. Kemble Ave. Morristown, N.J. 07962. Metoprolol is also commercially available under the brand name Toprol XL®, manufactured by Astra Zeneca, LP.

[0631] In one embodiment, mitochondrial-targeted creatine compounds of the present technology may be combined with one or more additional therapies for the prevention or treatment of porphyria. Treatment for acute attacks of hepatic porphyria typically comprise, but are not limited to, the use of narcotic analgesics (*e.g.*, for abdominal pain), phenothiazines (*e.g.*, for nausea, vomiting, anxiety, and restlessness), chloral hydrate (*e.g.*, for insomnia), short-acting benzodiazepines (*e.g.*, for insomnia), carbohydrate loading, intravenous hemin (*e.g.*, lyophilized hematin, heme albumin, and heme arginate), allogenic liver transplantation, and liver-directed gene therapy. Treatment for porphyria cutanea tarda typically comprises, but is not limited to, discontinuing risk factors (*e.g.*, alcohol, estrogens, iron supplements), phlebotomy, and low-dose regimens of chloroquine or hydroxychloroquine. Treatment for erythropoietic cutaneous porphyrias typically comprise, but is not limited to, chronic transfusions (*e.g.*, for anemia), protection from sunlight, treatment of complicating bacterial infections, and bone marrow and cord blood transplantation. Treatment for EPP and XLP typically comprise, but is not limited to, sunlight avoidance, oral β-carotene, administration of an α-melanocyte stimulating hormone analog, administration of cholestyramine and other porphyrin absorbents (*e.g.*, activated charcoal), plasmapheresis, intravenous hemin, and liver transplantation.

[0632] In some embodiments, the mitochondrial-targeted creatine compound is administered in combination with one or more narcotic analgesics, phenothiazines, chloral hydrate, benzodiazepines, hemin, chloroquine, hydroxychloroquine, β-carotene, α-melanocyte stimulating hormone, cholestyramine, or activated charcoal, such that a synergistic effect in the prevention or treatment of porphyria results.

[0633] In one embodiment, mitochondrial-targeted creatine compounds of the present technology may be combined with one or more additional therapies for the prevention or treatment of Alport Syndrome. Treatment for Alport Syndrome typically comprises, but are not limited to, the use of ACE inhibitors, ARBs, HMG-CoA reductase inhibitors, aldosterone inhibitors, aliskiren, calcineurin inhibitors (*e.g.*, cyclosporine A, tacrolimus), endothelin receptor antagonists (*e.g.*, sitaxentan, ambrisentan (LETAIRIS), atrasentan, BQ-123, zibotentan, bosentan (TRACLEER), macitentan, tezosentan, BQ-788 and A192621), sulodexide, vasopeptidase inhibitors (*e.g.*, AVE7688), anti-transforming growth factor-β1 antibody, chemokine receptor 1 blockers, bone morphogenetic protein-7, PPARγ agonists (*e.g.*, rosiglitazone, pioglitazone, MRL24, Fmoc-L -Leu, SR1664, SR1824, GW0072, MCC555, CLX-0921, PAT5A, L-764406, nTZDpa, CDDO (2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid), ragaglitazar, *O*-arylmandelic acids, and NSAIDs) and BAY-12-9566.

[0634] In some embodiments, the ACE inhibitors are selected from the group consisting of captopril, alacepril, lisinopril, imidapril, quinapril, temocapril, delapril, benazepril, cilazapril, trandolapril, enalapril, ceronapril, fosinopril, imadapril, mobertpril, perindopril, ramipril, spirapril, randolapril and pharmaceutically acceptable salts of such compounds.

[0635] In some embodiments, the ARBs are selected from the group consisting of losartan, candesartan, valsartan, eprosartan, telmisartan, and irbesartan.

[0636] In some embodiments, the HMG-CoA reductase inhibitors (or statins) are selected from the group consisting of lovastatin (*e.g.*, ADVICOR® (niacin extended-release/lovastatin) (AbbVie Pharmaceuticals, Chicago, Illinois), ALTOPREV™ (lovastatin extended-release) (Shiongi, Inc., Atlanta, GA), MEVACOR® (Merck, Whitehouse Station, NJ), atorvastatin (*e.g.*, CADUET® (amlodipine and atorvastatin) (Pfizer, Morrisville, PA), LIPITOR® (Pfizer, Morrisville, PA)), rosuvastatin and/or rosuvastatin calcium (*e.g.*, CRESTOR® (AstraZeneca, London, England)), simvastatin (*e.g.*, JUVISYNC® (sitagliptin/simvastatin) (Merck, Whitehouse Station, NJ)), SIMCOR® (niacin extended-release/simvastatin) (AbbVie Pharmaceuticals, Chicago, Illinois), VYTORIN® (ezetimibe/simvastatin) (Merck, Whitehouse Station, NJ), and ZOCOR® (Merck, Whitehouse Station, NJ)), fluvastatin and/or fluvastatin sodium (*e.g.*, LESCOL®, LESCOL XL (fluvastatin extended-release) (Mylan Pharmaceuticals, Morgantown, WV)), pitavastatin (*e.g.*, LIVALO® (Kowa Pharmaceuticals, Montgomery, AL)), pravastatin and pravastatin sodium (*e.g.*, PRAVACHOL® (Bristol-Myers Squibb, New York, NY)).

[0637] In some embodiments, the aldosterone inhibitors are selected from the group consisting of spironolactone (Aldactone®), eplerenone (Inspra®), canrenone (canrenoate potassium), prorenone (prorenoate potassium), and mexrenone (mexrenoate potassium).

[0638] In some embodiments, the mitochondrial-targeted creatine compound is administered in combination with one or more ACE inhibitors, ARBs, HMG-CoA reductase inhibitors, aldosterone inhibitors, aliskiren, calcineurin inhibitors (*e.g.*, cyclosporine A, tacrolimus), endothelin receptor antagonists (*e.g.*, sitaxentan, ambrisentan (LETAIRIS), atrasentan, BQ-123, zibotentan, bosentan (TRACLEER), macitentan, tezosentan, BQ-788 and A192621), sulodexide, vasopeptidase inhibitors (*e.g.*, AVE7688), anti-transforming growth factor-β1 antibody, chemokine receptor 1 blockers, bone morphogenetic protein-7, PPARγ agonists (*e.g.*, rosiglitazone, pioglitazone, MRL24, Fmoc-L -Leu, SR1664, SR1824, GW0072, MCC555, CLX-0921, PAT5A, L-764406, nTZDpa, CDDO (2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid), ragaglitazar, *O*-arylmandelic acids, and NSAIDs) and/or BAY-12-9566, such that a synergistic effect in the prevention or treatment of Alport Syndrome results.

[0639] In one embodiment, mitochondrial-targeted creatine compounds of the present technology may be combined with one or more additional therapies for the prevention or treatment of vitiligo. Treatment for vitiligo typically comprises, but is not limited to, the use of topical steroid creams, monobenzone, antibiotics, vitamins, hormones, immunomodulators, dermatologic drugs, or administering psoralen photochemotherapy. In some embodiments, the mitochondrial-targeted creatine compound is administered in combination with one or more topical steroid creams, monobenzone, antibiotics, vitamins, hormones, immunomodulators, dermatologic drugs, psoralen photochemotherapy, such that a synergistic effect in the prevention or treatment of vitiligo results.

[0640] In one embodiment, mitochondrial-targeted creatine compounds of the present technology may be combined with one or more additional therapies for the prevention or treatment of IPF. Additional therapies include, but are not limited to, oral corticosteroids, azathioprine, N-acetylcysteine, soluble human TNF receptor, interferon-γ1b therapy, endothelin receptor antagonists, phosphodiesterase inhibitors, tyrosine kinase inhibitors, antifibrotic agents, colchicine, and anticoagulants.

EXAMPLES

[0641] The present technology is further illustrated by the following examples, which should not be construed as limiting in any way.

Example 1 – Use of Mitochondrial-Targeted Creatine Compounds in the Treatment of Porphyria

[0642] This Example demonstrates the use of mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, in the treatment of porphyria.

[0643] Subjects suspected of having or diagnosed as having porphyria receive daily administrations of a therapeutically effective amount of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, alone or in combination with one or more additional agents for the treatment or prevention of porphyria. Mitochondrial-targeted creatine compounds and/or additional agents are administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly according to methods known in the art. Subjects will be evaluated weekly for the presence and/or severity of signs and symptoms associated with porphyria, including, but not limited to, *e.g.*, cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, life threatening acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension. Treatments are maintained until such a time as one or more signs or symptoms of porphyria are ameliorated or eliminated.

[0644] It is predicted that subjects suspected of having or diagnosed as having porphyria and receiving therapeutically effective amounts of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, will display reduced severity or elimination of one or more symptoms associated with porphyria. It is further expected that administration of mitochondrial-targeted creatine compounds in combination with one or more additional agents will have synergistic effects in this regard.

[0645] These results will show that mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, are useful in the treatment of porphyria.

Accordingly, the mitochondrial-targeted creatine compounds are useful in methods comprising administering mitochondrial-targeted creatine compounds to a subject in need thereof for the treatment of porphyria.

Example 2 – Use of Mitochondrial-Targeted Creatine Compounds in the Prevention of Porphyria

[0646] This Example demonstrates the use of mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, in the prevention of porphyria.

[0647] Subjects at risk of having porphyria receive daily administrations of a therapeutically effective amount of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, alone or in combination with one or more additional agents for the treatment or prevention of porphyria. Mitochondrial-targeted creatine compounds and/or additional agents are administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly according to methods known in the art. Subjects will be evaluated weekly for the presence and/or severity of signs and symptoms associated with porphyria, including, but not limited to, *e.g.*, cutaneous lesions, blistering skin lesions, hypertrichosis, hyperpigmentation, thickening and/or scarring of the skin, friability of the skin, photosensitivity of the skin, lichenification, leathery pseudovesicles, labial grooving, nail changes, life threatening acute neurological attacks, abdominal pain and cramping, constipation, diarrhea, increased bowel sounds, decreased bowel sounds, nausea, vomiting, tachycardia, hypertension, headache, mental symptoms, extremity pain, neck pain, chest pain, muscle weakness, sensory loss, tremors, sweating, dysuria, and bladder distension.

[0648] It is predicted that subjects at risk of having or diagnosed as having porphyria and receiving therapeutically effective amounts of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, will display delayed onset of porphyria, or prevention of onset of porphyria. It is further expected that administration of mitochondrial-targeted creatine compounds in combination with one or more additional agents will have synergistic effects in this regard.

[0649] These results will show that mitochondrial-targeted creatine compounds or pharmaceutically acceptable salts thereof, are useful in the prevention of porphyria.

Accordingly, the mitochondrial-targeted creatine compounds are useful in methods comprising administering mitochondrial-targeted creatine compounds to a subject in need thereof for the prevention of porphyria.

Example 3 – Use of Mitochondrial-Targeted Creatine Compounds in Treating IPF

[0650] *TGF-β1-adenovirus induced lung fibrosis:* TGF-β1 adenovirus (Ad-TGF- β1) or control virus (Ad-DL) is prepared and treated as previously described in Sime *et al.*, *J Clin Invest* 100:768-776 (1997). Ad-TGF-β1 refers to porcine TGF-β1 adenovirus (Ad-TGFβ1$^{223/225}$), an adenovirus construct containing a mutation of cysteine to serine at positions 223 and 225, rendering the expressed TGF-β1 biologically active. This virus expresses active TGF-β1 in the lung over a period of 7 to 14 days and produces extensive and progressive fibrosis in rats and mice. C57/B6 mice will receive 2 x 10^8 PFU virus in 50 µL sterile saline intratracheally and will be treated with saline; or a mitochondrial-targeted creatine compound. Mice are culled 5 or 14 days post instillation.

[0651] *Determination of lung fibrosis and inflammation:* Collagen content in the left lung is determined by sircol assay as per manufacturer's instructions. Histological lung inflammation and fibrosis score is carried out in Masson's trichrome stained sections.

[0652] Isolation of murine primary lung fibroblasts and primary type II alveolar epithelial cells: Primary cultures of lung fibroblasts are isolated by collagenase digestion (0.5 mg/ml for 1 hour at 37°C) of minced lungs and digests passed through a 100-µm cell strainer. Cells are cultured in DMEM containing 10% FCS for 4 days until confluent. Lung fibroblasts are used at passage 2. Lung alveolar epithelial cells (AECs) are extracted following the method originally described by Corti *et al.*, *Am J Respir Cell Mol Biol* 14:309-315 (1996). Immunofluorescence is carried out using the following primary antibodies: mouse monoclonal anti-α-SMA clone 1A4 (Sigma, Poole, UK), rabbit anti-mouse collagen 1 and mouse anti-active (ABC) β-catenin (Millipore).

[0653] *Results*: It is expected that intratracheal administration of adenoviral TGF-β1 (Ad-TGF-β1) in saline-treated mice will stimulate the formation of fibroblast foci, whereas mice treated with the Ad-DL control virus will not exhibit pulmonary fibrosis. Additionally, AECs from saline-treated Ad-TGF-β1 mice will show an increase in α-SMA and collagen-1 expression levels compared to that observed in AECs isolated from Ad-DL infected mice. It is also anticipated that Ad-TGF- β1 mice treated with the mitochondrial-targeted creatine

compounds will show a decrease in lung fibrosis and/or reduced collagen-1 and α-SMA levels compared to Ad-TGF-β1 mice treated with saline only.

[0654] These results will show that mitochondrial-targeted creatine compounds of the present technology are useful in treating IPF in mammalian subjects.

Example 4 – Use of Mitochondrial-Targeted Creatine Compounds to Reduce Tumor Growth

[0655] This Example will demonstrate use of the mitochondrial-targeted creatine compounds of the present technology to reduce the growth rate of implanted tumors.

[0656] A standard panel of 12 tumor cell lines will be used for the hollow fiber screening of the mitochondrial-targeted creatine compounds. These include NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295. The cell lines are cultivated in RPMI-1640 containing 10% FBS and 2 mM glutamine. On the day preceding hollow fiber preparation, the cells are given a supplementation of fresh medium to maintain log phase growth. For fiber preparation, the cells are harvested by standard trypsinization technique and resuspended at the desired cell density (2-10 x 10^6 cells/mL). The cell suspension is then flushed into 1 mm (internal diameter) polyvinylidene fluoride hollow fibers with a molecular weight exclusion of 500,000 Da. The hollow fibers are heat-sealed at 2 cm intervals and the samples generated from these seals are placed into tissue culture medium and incubated at 37 in 5% CO_2 for 24-48 hours prior to implantation. A total of 3 different tumor lines are prepared for each experiment so that each mouse receives 3 intraperitoneal implants (1 of each tumor line) and 3 subcutaneous implants (1 of each tumor line). On the day of implantation, samples of each tumor cell line preparation are quantitated for viable cell mass by a stable endpoint MTT assay so that the time zero cell mass is known. Mice are treated with vehicle or mitochondrial-targeted creatine compounds starting on day 3 or 4 following fiber implantation and continuing daily for 4 days. Control animals receive the tumor implants and are treated with only the empty vehicle. The therapeutic compositions are administered by intraperitoneal injection at 2 dose levels. The doses are based on the maximum tolerated dose (MTD) determined during prior toxicity testing. The fibers are collected from the mice on the day following the fourth compound treatment and subjected to the stable endpoint MTT assay. The optical density of each implanted tumor sample is determined spectrophotometrically at 540 nm and the mean of each treatment group is calculated. The percent net growth for each cell line in each treatment group is calculated and compared to

the percent net growth in the vehicle treated controls. A 50% or greater reduction in percent net growth in the treated samples compared to the vehicle control samples is considered a positive result. Each positive result is given a score of 2 and all of the scores are totaled for a given mitochondrial-targeted creatine compound. The maximum possible score for an agent is 96 (12 cell lines X 2 sites X 2 dose levels X 2 [score]). A compound is considered for xenograft testing if it has a combined ip + sc score of 20 or greater, a sc score of 8 or greater, or produces cell kill of any cell line at either dose level evaluated.

[0657] *Results*: It is expected that vehicle treated controls will show an increase in tumor net growth after 4 days. It is also anticipated that treatment with the mitochondrial-targeted creatine compounds will result in significantly reduced tumor net growth compared to vehicle treated controls.

[0658] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for reducing tumor growth in mammalian subjects. The results will show that the mitochondrial-targeted creatine compounds of the present technology are generally useful in treating a neoplastic disease.

Example 5 – Mitochondrial-Targeted Creatine Compounds Inhibit HUVEC Cell Migration

[0659] Chemotaxis is an integral part of angiogenesis, and this Example demonstrates the effect of mitochondrial-targeted creatine compounds of the present technology in inhibiting angiogenesis.

[0660] In the first portion of the experimental series, the effect of the chemoattractant vascular endothelial growth factor (VEGF) on human umbilical vein endothelial cells (HUVEC) is quantified. The experiment is carried out in a transwell plate, and in preparation therefor, HUVEC cells are grown to approximately 80% confluency. The cells are suspended in basal media and placed in a transwell plate on fibronectin coated membrane inserts at 50,000 cells per insert. Varying concentrations of VEGF are added to the bottom chamber of the transwell plate, and the plates are incubated for 4 hours at 37°C with a 5% CO_2 atmosphere. Following incubation, the membranes are fixed and stained. Nonmigrated cells are removed by mechanical abrasion and cells that migrate through the membrane are counted.

[0661] It is anticipated that VEGF will act as a chemotactic agent that induces cell migration, a process that is crucial to angiogenesis. Specifically, it is expected that certain VEGF concentrations will produce a strong chemotactic effect.

[0662] In the second portion of the experiment, the effect of mitochondrial-targeted creatine compounds in moderating chemotaxis, and hence angiogenesis, will be evaluated.

[0663] In this experimental series, HUVEC cells are incubated in a transwell plate with 30 ng/ml VEGF, and varying concentrations of the mitochondrial-targeted creatine compounds, under experimental conditions as described above. Group A cells will be incubated with VEGF only (positive control); Group B cells will be incubated with VEGF and a mitochondrial-targeted creatine compound; Group C will be incubated with VEGF-deficient growth medium only (negative control).

[0664] It is anticipated that cells incubated with VEGF alone will show an increase in cell migration compared to cells incubated with VEGF-deficient growth medium. It is also anticipated that the cells treated with mitochondrial-targeted creatine compounds will show a decrease in VEGF-mediated cell migration compared to the Group A positive control cells.

[0665] These results will show that mitochondrial-targeted creatine compounds of the present technology are useful as potent inhibitors of the angiogenic process, and as such will have utility in the treatment of diseases in which angiogenesis is a factor.

Example 6 – Therapeutic Effect of Mitochondrial-Targeted Creatine Compounds on 4-tertiary Butyl Phenol (4-TBP)-induced Cytotoxicity and Apoptosis in Melanocytes

[0666] This Example will demonstrate the therapeutic effect of mitochondrial-targeted creatine compounds on 4-TBP-induced vitiligo.

[0667] Melanocytes are cultured and treated with 4-TBP to induce vitiligo according to the procedures described in Yang & Boissy, *Pigment Cell Research*, 12:237-245 (1999). The experimental group of melanocytes is treated with 1-10 μg of a mitochondrial-targeted creatine compound after exposure to 4-TBP. The control melanocyte group is exposed to 4-TBP only.

[0668] It is anticipated that untreated melanocytes will exhibit high levels of cytotoxicity and apoptosis following exposure to 4-TBP (Vitiligo control) compared to melanocytes that

are not exposed to 4-TBP (Normal). However, it is anticipated that melanocytes treated with a mitochondrial-targeted creatine compound will show cell survival rates that are similar to normal melanocytes that are not exposed to 4-TBP and greater than untreated melanocytes following 4-TBP exposure.

[0669] These results will show that mitochondrial-targeted creatine compounds of the present technology, or pharmaceutically acceptable salts thereof, are useful in treating apoptosis and cytotoxicity associated with chemically-induced vitiligo. Accordingly, the mitochondrial-targeted creatine compounds of the present technology, are useful in treating, or ameliorating melanocyte degeneration and depigmentation observed in a subject suffering from or predisposed to vitiligo.

Example 7 – Use of Mitochondrial-Targeted Creatine Compounds in the Treatment of Alport Syndrome in Humans

[0670] This Example demonstrates the use of mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, in the treatment of Alport Syndrome.

[0671] Subjects suspected of having or diagnosed as having Alport Syndrome receive daily administrations of 1%, 5% or 10% solution of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, alone or in combination with one or more additional agents for the treatment or prevention of Alport Syndrome. Mitochondrial-targeted creatine compounds and/or additional agents are administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly according to methods known in the art. Subjects will be evaluated weekly for the presence and/or severity of signs and symptoms associated with Alport Syndrome, including, but not limited to, *e.g.*, hematuria, proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, Glomerular Basement Membrane (GBM) ultrastructural abnormalities, nephrotic syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis. Treatments are maintained until such a time as one or more signs or symptoms of Alport Syndrome are ameliorated or eliminated.

[0672] It is predicted that subjects suspected of having or diagnosed as having Alport Syndrome and receiving therapeutically effective amounts of a mitochondrial-targeted creatine compound, or a pharmaceutically acceptable salt thereof, will display reduced severity or elimination of one or more symptoms associated with Alport Syndrome. It is also expected that Alport Syndrome subjects treated with the mitochondrial-targeted creatine compound will show normalization of one or more of ADAM8, fibronectin, myosin 10, MMP-2, MMP-9, and podocin urine levels by at least 10% compared to the untreated Alport Syndrome controls. It is further expected that administration of mitochondrial-targeted creatine compounds in combination with one or more additional agents will have synergistic effects in this regard compared to that observed in subjects treated with the mitochondrial-targeted creatine compounds or the additional agents alone.

[0673] These results will show that mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, are useful in the treatment of Alport Syndrome. These results will show that mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, are useful in ameliorating one or more of the following symptoms: hematuria, proteinuria, cylindruria, leukocyturia, hypertension, edema, microalbuminuria, declining glomerular filtration rate, fibrosis, GBM ultrastructural abnormalities, nephrotic syndrome, glomerulonephritis, end-stage kidney disease, chronic anemia, macrothrombocytopenia, osteodystrophy, sensorineural deafness, anterior lenticonus, dot-and-fleck retinopathy, posterior polymorphous corneal dystrophy, recurrent corneal erosion, temporal macular thinning, cataracts, lacrimation, photophobia, vision loss, keratoconus, and leiomyomatosis. Accordingly, the mitochondrial-targeted creatine compounds are useful in methods comprising administering mitochondrial-targeted creatine compounds to a subject in need thereof for the treatment of Alport Syndrome.

Example 8 – Use of Mitochondrial-Targeted Creatine Compounds in the Prevention of Leber's Hereditary Optic Neuropathy (LHON) in a Mouse Model

[0674] This Example will demonstrate the use of mitochondrial-targeted creatine compounds in the prevention of Leber's Hereditary Optic Neuropathy in a mouse model.

[0675] *Murine Model.* This Example uses the murine model of LHON previously described by Lin *et al.*, *Proc. Natl. Acad. Sci.* 109(49):20065-20070 (2012). The animals harbor an ND6 P25L mutation. The LT13 cell line corresponds to the ND6 P25L mutant fibroblast line used for mouse embryonic stem cell fusions.

[0676] Mice harboring the ND6 P25L mutation are administered 1-10 μg of a mitochondrial-targeted creatine compound, or saline vehicle (control) subcutaneously once daily from 0-14 months of age. Various aspects of LHON are assessed in treatment and control animals at 14 and 24 months of age, with the ND6 P25L compared to wild-type mice for each parameter measured.

[0677] It is expected that administration of mitochondrial-targeted creatine compounds once daily from 0-14 months of age will prevent the onset of, delay the onset of, and/or reduce the severity of the effects of the ND6 P25L mutation, thereby preventing LHON in ND6 P25L mutant mice.

[0678] *Reduced Retinal Response.* The ND6 P25L mice are examined for ocular function by electroretinogram beginning at 14 months of age. It is expected that the animals will show a significant deficit in nearly all parameters examined. The scotopic B wave of dark-adapted ND6 P25L eyes is expected to be reduced in amplitude by approximately 25.5% and approximately 33.1% with 0.01 and 1 cd·s/m^2 (maximum) stimulations. The scotopic A-wave of ND6 P25L mutant eyes is expected to show approximately a 23% reduction. The scotopic oscillatory potentials (OPs), a high-frequency response derived from multiple retinal cell types, are expected to show approximately a 20.7% and approximately a 21.7% reduction with 0.01 and 1 cd·s/m^2 stimulations. Photopic B-wave ERG amplitude, measuring cone functions, is expected to be decreased approximately 17.7%. There is further expected a trend toward increased latencies to the A and B waves. Despite the functional deficit observed in the ERGs, it is expected that the ND6 P25L mutants will not exhibit reduced visual responses, as assessed by optokinetic analysis.

[0679] It is expected that administration of mitochondrial-targeted creatine compounds once daily from 0-14 months of age will prevent the onset of, delay the onset of, and/or reduce the severity of these effects of the ND6 P25L mutation, thereby preventing these aspects of LHON in ND6 P25L mutant mice.

[0680] *RGC Axonal Swelling and Preferential Loss of Smallest Fibers.* Electron microscopic analysis of RGC axons is expected to reveal that ND6 P25L mutants exhibit axonal swelling in the optic nerve. The average axonal diameter is expected to be approximately 0.67 μm in wild-type and approximately 0.80 μm in ND6 P25L mutant 14-month-old mice, and approximately 0.73 μm in wild-type and approximately 0.85 μm in ND6

P25L mutant mice at 24 months of age. Fourteen-month-old ND6 P25L mutant mice are expected to have an increased number of large fibers but fewer small axonal fibers (=0.5 μm). The change in axonal diameters is expected to be more pronounced in 24-month-old ND6 P25L mice. Hence, ND6 P25L mice are expected to have fewer small and medium axons (=0.8 μm) and more swollen axonal fibers with diameters larger than 1 μm. This effect is expected to be the most severe in the area of the smallest fibers in the central and temporal regions of the mouse optic nerve, which corresponds to the human temporal region most affected in LHON.

[0681] Quantification of the number of axons in the optic nerves is expected to reveal no significant difference in the total counts at 14 months of age, and approximately a 30% reduction at 24 months of age. Thus, the observed shift toward larger axons is predicted to be attributable initially (14 months) to swelling of medium axons, and later (24 months), to the loss of small axons.

[0682] It is expected that administration of mitochondrial-targeted creatine compounds once daily from 0-14 months of age will prevent the onset of, delay the onset of, or reduce the severity of these effects in ND6 P25L mutant animals, thereby preventing these aspects of LHON.

[0683] *Abnormal Mitochondrial Morphology and Proliferation in RGC Axons.* Mitochondria in the optic tracts of the ND6 P25L mutants are expected to be abnormal and increased in number, consistent with the compensatory mitochondrial proliferation observed in LHON patients. The optic tract axons of 14-month-old ND6 P25L mice are expected to have approximately a 58% increase in mitochondria, with 24-month-old animals having approximately a 94% increase. The ND6 P25L mitochondria are expected to appear hollowed with irregular cristae, with approximately 31.5% more of the ND6 P25L mitochondria being abnormal at 14 months and approximately 56% more at 24 months of age. Axons filled with abnormal mitochondria are expected to demonstrate marked thinning of the myelin sheath.

[0684] It is expected that administration of mitochondrial-targeted creatine compounds once daily from 0-14 months of age will prevent the onset of, delay the onset of, or reduce the severity of these effects in ND6 P25L mutant animals, thereby preventing these aspects of LHON.

[0685] *Altered Liver Mitochondria Complex I Activity.* The complex I activity of the ND6 P25L mice is assayed in liver mitochondria. Results are expected to demonstrate that rotenone-sensitive NADH:ubiquinone oxidoreductase activity is decreased by approximately 29%, which is equivalent to the reduction seen in the LT13 cell line. It is expected that the decrease in activity will not be attributable to a lower abundance of complex I, as it is expected that the NADH:ferricyanide oxidoreductase will be unaltered in the ND6 mutant mice. It is further expected that the ND6 mutation will cause approximately a 25% decrease in mitochondrial oxygen consumption, also seen in the LT13 cell line.

[0686] It is expected that administration of mitochondrial-targeted creatine compounds once daily from 0-14 months of age will prevent the onset of, delay the onset of, or reduce the severity of these effects in ND6 P25L mutant animals, thereby preventing these aspects of LHON.

[0687] These results will show that mitochondrial-targeted creatine compounds of the present technology, are useful for preventing the onset of, delaying the onset of, and/or reducing the severity of the symptoms of LHON in a mammalian subject. As such, mitochondrial-targeted creatine compounds of the present technology are useful in methods for preventing LHON in a mammalian subject.

Example 9 – Use of Mitochondrial-Targeted Creatine Compounds in Treating Friedreich's Ataxia in *in vitro* Cell Culture

[0688] This Example will demonstrate the use of mitochondrial-targeted creatine compounds of the present technology in the treatment of Friedreich's Ataxia in a cell culture model of the disease.

[0689] *Cell line model.* This Example uses human dermal fibroblasts derived from Friedreich's Ataxia patients previously described by Jauslin *et al.*, *Hum. Mol. Genet.* 11(24):3055 (2002).

[0690] Fibroblasts from Friedreich's Ataxia (FRDA) patients have been shown to be hypersensitive to L-buthionine-(S,R)-sulfoximine (BSO), a specific inhibitor of GSH synthetase. Jauslin *et al.*, *Hum. Mol. Genet.* 11(24):3055 (2002), Jauslin *et al.*, *FASEB J.* 17:1972-4 (2003), and International Patent Application WO 2004/003565. The therapeutic

efficacy of a compound can be assessed by assaying its ability to suppress BSO-mediated cell death in FRDA fibroblasts.

[0691] FRDA fibroblasts and fibroblasts from normal subjects are seeded in microtiter plates at a density of 4000 cells per 100 µL in growth medium consisting of 25% (v/v) M199 EBS and 64% (v/v) MEM EBS without phenol red (Bioconcept, Allschwil, Switzerland) supplemented with 10% (v/v) fetal calf serum (PAA Laboratories, Linz, Austria), 100 U/mL penicillin, 100 µg/mL streptomycin (PAA Laboratories, Linz, Austria), 10 µg/mL insulin (Sigma, Buchs, Switzerland), 10 ng/mL EGF (Sigma, Buchs, Switzerland), 10 ng/mL bFGF (PreproTech, Rocky Hill, NJ, USA) and 2 mM glutamine (Sigma, Buchs, Switzerland).

[0692] The test samples are supplied in 1.5 ml glass vials. The mitochondrial-targeted creatine compounds are diluted with DMSO, ethanol or PBS to result in a 5 mM stock solution. Once dissolved, they are stored at −20° C. Reference antioxidants (Idebenone, decylubiquinone, α-tocopherol acetate and trolox) are dissolved in DMSO.

[0693] Test samples are screened according to the following protocol:

[0694] A culture with FRDA fibroblasts is started from a 1 ml vial with approximately 500,000 cells stored in liquid nitrogen. Cells are propagated in 10 cm cell culture dishes by splitting every third day in a ratio of 1:3 until nine plates are available. Once confluent, fibroblasts are harvested. For 54 micro titer plates (96 well-MTP) a total of 14.3 million cells (passage eight) are re-suspended in 480 ml medium, corresponding to 100 µl medium with 3,000 cells/well. The remaining cells are distributed in 10 cm cell culture plates (500,000 cells/plate) for propagation. The plates are incubated overnight at 37° C. in an atmosphere with 95% humidity and 5% CO_2 to allow attachment of the cells to the culture plate.

[0695] MTP medium (243 µl) is added to a well of the microtiter plate. The mitochondrial-targeted creatine compounds are thawed, and 7.5 µl of a 5 mM stock solution is dissolved in the well containing 243 µl medium, resulting in a 150 µM master solution. Serial dilutions from the master solution are made. The period between the single dilution steps is kept as short as possible (generally less than 1 second).

[0696] Plates are kept overnight in the cell culture incubator. The next day, 10 µl of a 10 mM BSO solution is added to the wells, resulting in a 1 mM final BSO concentration. Forty-eight hours later, three plates are examined under a phase-contrast microscope to verify that

the cells in the 0% control (wells E1-H1) are clearly dead. The medium from all plates is discarded, and the remaining liquid is removed by gently tapping the plate inversed onto a paper towel.

[0697] 100 µl of PBS containing 1.2 µM Calcein AM is then added to each well. The plates are incubated for 50-70 minutes at room temperature. Then, the PBS is discarded, and the plate is gently tapped on a paper towel. Fluorescence intensity is measured with a Gemini Spectramax XS spectrofluorimeter (Molecular Devices, Sunnyvale, CA, USA) using excitation and emission wavelengths of 485 and 525 nm, respectively.

[0698] It is anticipated that untreated FRDA cells will exhibit high levels of cell death following exposure to BSO (FRDA Control) as compared to fibroblasts derived from normal subjects (Normal). However, it is anticipated that FRDA fibroblasts treated with mitochondrial-targeted creatine compounds will show cell survival rates that are similar to normal subjects and greater than untreated FRDA fibroblasts, following BSO exposure.

[0699] These results will show that mitochondrial-targeted creatine compounds of the present technology are useful in the treatment of Friedreich's Ataxia.

Example 10 – Use of Mitochondrial-Targeted Creatine Compounds in Treating Mitochondrial Iron Loading in Friedreich's Ataxia Mouse Model

[0700] This Example will demonstrate the use of mitochondrial-targeted creatine compounds of the present technology in treating mitochondrial iron loading in a mouse model of Friedreich's Ataxia.

[0701] *Mouse model.* This Example uses the muscle creatine kinase (MCK) conditional frataxin knockout mice described by Puccio *et al.*, *Nat. Genet.* 27:181-186 (2001). In this model, the tissue-specific Cre transgene under the control of MCK promoter results in the conditional deletion of frataxin in only the heart and skeletal muscle.

[0702] 8-week-old mutant mice are administered a daily dose of 0.25 mg/kg/day of a mitochondrial-targeted creatine compound, or saline vehicle only (control) subcutaneously for two weeks. Total RNA is isolated from hearts of two 10-week-old wild-type mice, two 10-week-old untreated mutant mice and two 10-week-old treated mutant mice. Total RNA is isolated using TRIzol (Invitrogen). First-strand cDNA synthesis and biotin-labeled cRNA are performed and hybridized to the mouse Affymetrix GeneChip 430 2.0. A 2-phase strategy is

used to identify differentially expressed genes. First, genome-wide screening is performed using Affymetrix GeneChips. Then, low-level analysis is performed with Affymetrix GeneChip Operating Software 1.3.0, followed by the GC robust multiarray average (GCRMA) method for background correction and quantile–quantile normalization of expression. Tukey's method for multiple pairwise comparisons is applied to acquire fold-change estimations. Tests for significance are calculated and adjusted for multiple comparisons by controlling the false discovery rate at 5%.

[0703] Definitive evidence of differential expression is obtained from RT-PCR assessment of samples used for the microarray analysis and at least 3 other independent samples. Principal component analysis is performed by standard methods. Western blot analysis is performed using antibodies against frataxin (US Biological); Tfr1 (Invitrogen); Fpn1 (D. Haile, University of Texas Health Science Center); Hmox1 (AssayDesigns); Sdha, Gapdh, and Iscu1/2 (Santa Cruz Biotechnology); Fech (H. Dailey, University of Georgia, Biomedical and Health Sciences Institute); Hfe2 (S. Parkkila, University of Tampere, Institute of Medical Technology); Nfs1, Uros, and Alad (Abnova); Sec15l1 (N.C. Andrews, Duke University); Ftl1, Fth1, Ftmt (S. Levi, San Raffaele Institute); and Hif1α (BD Biosciences).

[0704] For heme assays, hearts are exhaustively perfused and washed with PBS (0.2% heparin at 37 °C) to remove blood. After homogenization, heme is quantified using the QuantiChrom Heme Assay (BioAssay Systems). Tissue iron is measured *via* inductively coupled plasma atomic emission spectrometry.

[0705] It is anticipated that untreated mutant mice will exhibit decreased expression of genes involved in heme synthesis, iron–sulfur cluster assembly, and iron storage (FRDA Control) as compared to wild-type mice (Normal). However, it is anticipated that mutant mice treated with the mitochondrial-targeted creatine compounds will show expression levels that are similar to normal subjects with respect to genes involved in these three mitochondrial iron utilization pathways.

[0706] These results will show that mitochondrial-targeted creatine compounds of the present technology are useful in treating mitochondrial iron loading in a mammalian model of Friedreich's Ataxia.

Example 11 – Use of Mitochondrial-Targeted Creatine Compounds in Treating Complex I and ATP Content Deficiency in Patients Suffering from a Mitochondrial Disease or Disorder

[0707] This Example will demonstrate the use of mitochondrial-targeted creatine compounds of the present technology in treating complex I and ATP content deficiency in patients suffering from a mitochondrial disease or disorder.

[0708] Patients diagnosed with any mitochondrial disease or disorder described herein are administered a daily dose of 0.5 mg/kg/day of a mitochondrial-targeted creatine compound; or saline vehicle (control) for six weeks.

[0709] *Isolation of Lymphocytes from Peripheral Blood.* Blood is diluted with Hank's solution at a ratio of 1:2 within one hour of extraction and slowly layered onto a 15-mL screw-cap tube containing 5 mL Ficolymph (Bharafshan Co. Tehran, Iran.). The tubes are centrifuged for 20 minutes at 1000 × g, after which the lymphocyte-containing layer is collected into a new centrifuge tube using a sterile pipette. The lymphocyte mix is then diluted in 10 mL Hank's solution and centrifuged for 10 minutes at 440×g. The supernatant is discarded, 5 mL of Hank's solution is added, the pellet is mixed gently in this buffer, and the mixture is allowed to sit for about 45 seconds (s). The mixture is gently pipetted and then centrifuged at 230×g for 15 minutes. The supernatant is discarded, and the pellet is suspended in RPMI 1640 medium (Bharashan Co. Tehran, Iran) supplemented with L-glutamine.

[0710] *Complex I activity assay.* Fresh lymphocyte pellets are homogenized by sonication in 20 mmol/L potassium phosphate buffer (pH 7.5) for 15s (three bursts of 5s each) at 30W on ice. The final protein concentration is quantified according to Bradford's method. The homogenate, containing 2-4 g/L protein, is kept on ice and used for assay the same day. Biochemical studies are carried out on lymphocyte homogenate of 12 patients and 25 controls. NADH-ferricyanide reductase activity is also assayed spectrophotometrically by following the disappearance of oxidized ferricyanide at 410 nm and 30°C. The assay mixture contained in 1 mL: NADH, ferricyanide, triethanolamin and phosphate buffer (pH 7.8). The reaction is started by the addition of the lymphocyte homogenate.

[0711] *Extraction and quantification of intracellular ATP.* The lymphocyte cells are pelleted in a microcentrifuge tube by centrifugation at 12,000 g for 10 min. The cellular ATP is then extracted by adding 0.5 mL water and boiling the cell pellet for 5 min. After

vortexing and centrifugation (12,000 g for 5 min at 4°C), 50 μL of the supernatant is used for bioluminescence measurement. The standard curve of ATP is obtained by serial dilutions of 4 mM ATP solution (0.25, 0.5, 1.0, 2.0, and 4.0). Light emission is measured with a Sirius tube luminometer, Berthold defection system (Germany). After calibration against the ATP standard, the ATP content of the cell extract is determined.

[0712] It is anticipated that lymphocytes derived from untreated subjects will exhibit decreased complex I activity and reduced intracellular ATP levels (mitochondrial disease Control) as compared to controls (Normal). However, it is anticipated that subjects treated with the mitochondrial-targeted creatine compound will show complex I activity and ATP levels that are similar to normal subjects and greater than untreated subjects suffering from a mitochondrial disease or disorder.

[0713] These results will show that mitochondrial-targeted creatine compounds of the present technology are useful in treating complex I and ATP content deficiency in patients suffering from a mitochondrial disease or disorder.

Example 12 – Use of Mitochondrial-Targeted Creatine Compounds in Restoring Aconitase Activity in Cultured Cells Following Deferiprone Exposure

[0714] This Example will demonstrate the use of mitochondrial-targeted creatine compounds of the present technology in restoring aconitase activity in cultured cells that have been exposed to deferiprone.

[0715] Deferiprone has been shown to potently impair aconitase activity, presumably through reduced iron-sulfur cluster biosynthesis. Goncalves *et al.*, *BMC Neurology* 8:20 (2008).

[0716] Fibroblasts derived from forearm biopsies taken from healthy controls are grown under standard conditions in Dulbecco's modified Eagle's medium (DMEM; Gibco Invitrogen, Cergy Pontoise, France) supplemented with 10% fetal calf serum, 10 mg/mL penicillin/streptomycin and 2 mM L-Glutamine (as Glutamax™; Gibco Invitrogen). Final iron content in culture medium will amount to 2–3 μM. The medium (4 mL/25 cm^2 flask; 3 mL/10 cm^2 well) is changed every three days.

[0717] Fibroblasts are administered 1-10 μg of a mitochondrial-targeted creatine compound, or empty vehicle (control) for 24 hours before addition of deferiprone.

Fibroblasts are seeded at 18 × 103 cells/cm^2. Fibroblasts are then treated with 75 μM deferiprone for 7 days. Aconitase measurement is spectrophotometrically carried out by following aconitate production from citrate at 240 nm on the supernatant (800 g × 5 min) of detergent-treated cells (0.2% lauryl maltoside). Protein concentration is measured according to Bradford method.

[0718] It is anticipated that untreated fibroblasts will exhibit reduced aconitase activity following exposure to deferiprone (Control) as compared to fibroblasts that are not exposed to deferiprone (Normal). However, it is anticipated that concurrent treatment with the mitochondrial-targeted creatine compound will show aconitase activity that is similar to normal subjects and greater than untreated fibroblasts following deferiprone exposure.

[0719] These results will show that mitochondrial-targeted creatine compounds of the present technology are useful in restoring aconitase activity in cultured cells that have been exposed to deferiprone.

Example 13 – Use of Mitochondrial-Targeted Creatine Compounds in Reducing Mitochondrial Fission

[0720] This Example will demonstrate use of the mitochondrial-targeted creatine compounds of the present technology in the reduction of mitochondrial fission.

[0721] Cultured human SH-SY5Y neuronal cells are treated with buffer; 5 μM CCCP (carbonyl cyanide m-chloro phenyl hydrazone, a mitochondrial uncoupler); or 5 μM CCCP and a mitochondrial-targeted creatine compound; for 30 minutes. The cells are then stained with anti-Tom20 antibody, a mitochondrial marker, and Hoechst stain. Mitochondrial morphology is analyzed using 63X oil immersion lens.

[0722] *Results* – It is expected that control cells treated with CCCP will show extensive mitochondrial fragmentation as manifested by small, round or dot-like staining patterns. It is also anticipated that treatment with the mitochondrial-targeted creatine compound will result in significantly reduced mitochondrial fission compared to control cells that are only exposed to CCCP.

[0723] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for reducing mitochondrial fission in mammalian subjects.

Example 14 – Use of Mitochondrial-Targeted Creatine Compounds to Increase Protein Expression Levels of Fully Assembled Complex I and Complex II in Cells Bearing Complex I Mutations

[0724] This Example will demonstrate use of the mitochondrial-targeted creatine compounds of the present technology to restore electron transport chain function in complex I mutant cells.

[0725] Experimental fibroblast cells are derived from patients with a mutation in different Complex I subunits. Control cells are human skin fibroblasts derived from healthy controls. Cultured Complex I mutant fibroblasts are incubated with buffer or a mitochondrial-targeted creatine compound for up to 72 hours. The cells are then harvested by trypsinization and washed twice with ice-cold PBS. The cell suspensions are centrifuged for 5 minutes at 4 °C and the cell pellets are snap-frozen in liquid nitrogen. The cell pellets are subsequently thawed on ice and resuspended in 100 µl of ice-cold PBS.

[0726] *Isolation of OXPHOS complexes*: The cell suspension is incubated with 100 µL (4 mg/mL) digitonin (Sigma, Zwijndrecht, Netherlands) on ice for 10 min. Digitonin dissociates membranes that contain cholesterol, thereby dissociating the cell membrane and the outer mitochondrial membrane, but not the inner mitochondrial membrane. Next, 1 mL ice-cold PBS is added to dilute the digitonin, followed by centrifugation (10 min; 15,600xg; 4 °C). The resulting pellets contain a cell fraction which is enriched for mitoplasts. The supernatant is removed and the pellets are resuspended in 100 µL ice-cold PBS. 1 mL ice-cold PBS is then added and the suspension is centrifuged again (5 min; 15,600 xg; 4 °C), followed by removal of the supernatant and resuspension of the pellet in 100 µL ice-cold PBS. The supernatant is removed with a syringe and needle and the pellets containing the mitoplast fraction are stored overnight (-20 °C).

[0727] The complexes of the OXPHOS system are extracted from the inner membrane with β-lauryl maltoside and aminocaproic acid. The pellets are thawed on ice and solubilized in 100 µL ACBT buffer containing 1.5 M ε-aminocaproic acid (Serva, Amsterdam, Netherlands) and 75 mM Bis-Tris/HCl (pH 7.0) (Sigma). Subsequently 10 µL 20% (w/v) β-lauryl maltoside (Sigma) is added and the suspension is left on ice for 10 min. Next, the suspensions are centrifuged (30 min; 15,600 xg; 4 °C) and the supernatants which contain the isolated complexes are transferred to a clean tube (L.G. Nijtmans *et al.*, *Methods* 26 (4): 327-

334 (2002)). The protein concentration of the isolated OXPHOS complexes is determined using a Biorad Protein Assay (Biorad, Veenendaal, Netherlands). Blue-native PAGE analysis of mitoplasts is performed as described in L.G. Nijtmans *et al.*, *Methods* 26 (4): 327-334 (2002).

[0728] *Complex I or complex II protein detection*: To visualize the amount of complex I or complex II present in the BN-PAGE gels, the proteins are transferred to a PVDF membrane (Millipore, Amsterdam, Netherlands) using standard Western blotting techniques and detected by immunostaining. After the blotting and prior to blocking the PVDF membrane with 1:1 PBS-diluted Odyssey blocking buffer (Li-cor Biosciences, Cambridge, UK), the PVDF blot is stripped with stripping buffer for 15 min at 60°C. The stripping buffer consists of PBS, 0.1% Tween-20 (Sigma) and 2% SDS (Serva). A monoclonal primary antibody against NDUFA9 (39 kDa) (Molecular probes, Leiden, The Netherlands) is used for detection of Complex I. To detect Complex II, a monoclonal antibody against the 70 kDa subunit of complex II is used (Molecular probes). Both primary antibodies are diluted in PBS, 0.1% Tween-20 and 2.5% Protifar Plus (Nutricia, Cuijk, The Netherlands) and allowed to bind to the complex for 4 hours at room temperature or overnight at 4°C. The bound primary antibodies are subsequently detected by IRDye 800 CW conjugated anti-Mouse antibody (Li-cor Biosciences) at a final concentration of 0.1 μg/mL.

[0729] *Results*: It is expected that untreated Complex I mutant cells will show reduced protein expression levels of Complex I and Complex II compared to untreated healthy control cells. It is also anticipated that treatment with the mitochondrial-targeted creatine compound will result in an increase in fully assembled complex I and complex II protein levels in Complex I mutant cells.

[0730] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for elevating Complex I and Complex II protein levels in mammalian subjects. The results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in promoting electron transport chain function generally.

Example 15 – *In vivo* Effect of Mitochondrial-Targeted Creatine Compounds on Grip Strength in Ndufs4 Knockout Mice

[0731] This Example will demonstrate use of the mitochondrial-targeted creatine compounds of the present technology to improve grip strength in Ndufs4 knockout (Complex I deficient) mice.

[0732] *Animals and Treatments*: Ndufs4 knockout (KO) and wild-type (WT) mice are generated by crossing Ndufs4 heterozygote males and females (Kruse SE, *et al.*, 2008, *Cell Metab* 7:312-320). Animals are divided into the following groups: Vehicle-WT; Vehicle-KO; or mitochondrial-targeted creatine compound-KO. Animals are tested at 3, 5 and 6 weeks of age. Animals will receive either vehicle (control) injections, consisting of sterile water, or injections consisting of a mitochondrial-targeted creatine compound. Animals are injected twice a day. Injections begin during week 3 of life, and are continued daily until the conclusion of the experiment in week 6.

[0733] *Data Analysis*: All data are expressed as mean + SEM. Data are analyzed using a one way ANOVA in SPSS version 20.0. Significant overall effects (*i.e.*, genotype, treatment and/or genotype treatment interaction) are further analyzed using Fisher's PLSD post-hoc analyses.

[0734] *Grip Strength Paradigm*: The grip strength test is designed to measure muscular strength in rodents. The apparatus consists of a single bar, which the animal will grasp by instinct. Once the bar has been grasped, the experimenter gently retracts the animal until the animal is forced to release the bar. The amount of force exerted by the animal on the bar is measured in Pond (p) (1 p = 1 gram). The grip strength test is repeated 5 times and the average force exerted is used as the quantitative readout. All measurements will be corrected for body weight, using the following equation:

[0735] Grip Strength Score = ((week X trials 1 + 2 + 3 + 4 +5)/5)/ Average Body Weight week X (g) (Week X = week 3, 5 or 6)

[0736] Testing Procedure: On testing days, animals will receive their morning injection 30 minutes prior to their testing time. After injections, the animals will be placed in the testing room for a 30 minute acclimation period.

[0737] *Results*: It is expected that vehicle KO animals will show remarkably decreased grip strength compared to wild-type control animals. It is also anticipated that chronic treatment with the mitochondrial-targeted creatine compound will result in significantly improved grip strength in the knockout animals compared to vehicle knockouts.

[0738] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for improving grip strength in mammalian subjects. The results will show that the mitochondrial-targeted creatine compounds of the present technology are generally useful in treating neuromuscular defects in Complex I deficient subjects.

Example 16 – Mitochondrial-Targeted Creatine Compounds Restore Motor and Cognitive Function in an *in vivo* Huntington Disease (HD) Animal Model

[0739] This Example will demonstrate use of the mitochondrial-targeted creatine compounds of the present technology to reduce the neurological defects associated with HD.

[0740] R6/2 mice, expressing exon 1 of the human HD gene carrying more than 120 CAG repeats, exhibit progressive neurological phenotypes that mimic the features of HD in humans. The mice develop progressive neurological phenotypes gradually with mild phenotype (*e.g.*, resting tremor) as early as 5 weeks of age and severe symptoms (including reduced mobility and seizures) at 9–11 weeks, with many of the mice dying by 14 weeks.

[0741] R6/2 HD transgenic mice are treated with an empty vehicle or a mitochondrial-targeted creatine compound using Alzet osmotic mini-pumps from age 5 weeks to 13 weeks. These animals will be subjected to a number of behavioral assessments to study motor and cognitive function. Rotor-rod and mobility in an activity chamber are used for assessment of motor function, and the Y-maze is used for assessment of working memory.

[0742] *Results* – It is anticipated that vehicle-treated R6/2 mice will display major motor deficits such as a reduced ability to stand on their rear limbs and increased periods of immobility compared to wild-type controls. It is further anticipated that treatment with the mitochondrial-targeted creatine compound will restore motor activity and improve cognitive function (as demonstrated by the animals' performance in the Y-maze test).

[0743] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for restoring cognitive and motor function in

mammals suffering from HD. The results will show that the mitochondrial-targeted creatine compounds of the present technology are generally useful in treating symptoms associated with neurodegenerative diseases.

Example 17 – Use of Mitochondrial-Targeted Creatine Compounds to Suppress Aβ-mediated Toxicity in the Brain

[0744] This Example will demonstrate use of the mitochondrial-targeted creatine compounds of the present technology to treat or ameliorate the toxic effects of Aβ accumulation in brain tissue.

[0745] Rats are treated with saline or a mitochondrial-targeted creatine compound (0.5–2 μmol/kg body weight, n=12). The compositions are injected intraperitoneally into the animal 24 hours before hippocampal slices are obtained to measure long-term potentiation (LTP). Brain slices from each group are incubated with Aβ fragments for 15 min before evaluating LTP.

[0746] *Results* – It is expected that brain slices recovered from saline-treated controls will show impaired LTP post Aβ treatment. It is also anticipated that treatment with the mitochondrial-targeted creatine compound will suppress Aβ-mediated impairment of LTP.

[0747] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for treating or ameliorating Aβ-mediated toxicity in brain tissue. The results will show that the mitochondrial-targeted creatine compounds of the present technology are generally useful in reducing the synaptic dysfunction and memory loss caused by Aβ accumulation generally.

Example 18 – Use of Mitochondrial-Targeted Creatine Compounds to Delay Ageing

[0748] This Example will demonstrate use of the methods and compositions of the present technology to reduce the frequency and/or severity of age-related symptoms. The Example will demonstrate the use of mitochondrial-targeted creatine compounds of the present technology in delaying ageing.

[0749] Ercc1$^{-/\Delta}$ progeroid mice are treated with a mitochondrial-targeted creatine compound (i.p. about 0.5-2 mg/kg) in sunflower oil carrier three times per week over an 18-21 week period. Control animals are Ercc1$^{-/\Delta}$ progeroid mice that receive sunflower seed oil according

to the same schedule. The treated and control mice are monitored twice a week for weight and symptom/sign development. Symptoms include dystonia, trembling, kyphosis, ataxia, wasting, priapism, decreased activity, incontinence, and vision loss. The rate of deterioration of intervertebral discs (an index of degenerative disease of the vertebra) is assessed by measuring the level of glycosaminoglycan in the discs in treated and control mice.

[0750] *Results* – It is expected that treatment with the mitochondrial-targeted creatine compounds will result in a significant delay in onset of age-related degeneration compared to controls treated with vehicle only. It is also anticipated that the intervertebral discs of mice treated with the mitochondrial-targeted creatine compounds will contain more glycosaminoglycan relative to control mice, indicating inhibition of disc degeneration.

[0751] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for reducing the frequency and/or severity of age-related symptoms. The results will show that the methods and compositions described herein are useful in delaying ageing generally.

Example 19 – Use of Mitochondrial-Targeted Creatine Compounds to Treat Mitochondrial Dysfunction

[0752] This Example demonstrates the use of mitochondrial-targeted creatine compounds, or pharmaceutically acceptable salts thereof, to treat different aspects of mitochondrial dysfunction.

[0753] Lymphocytes, fibroblasts or neurons are derived from subjects suspected of having or diagnosed as having a mitochondrial disease or disorder. The isolated cells are cultured using conventional methods that promote optimal growth of a given cell type. The resulting cell cultures are subjected to the following assays:

[0754] *Mitochondrial Membrane Potential (Δψm) Assay*: For the determination of Δψm, cells are pre-treated with or without mitochondrial-targeted creatine compounds. The cells are treated with 5 mM Dulbecco's modified Eagle's minimal essential medium (DEM) for 120 minutes, collected by centrifugation at 300×g for 3 minutes and then washed twice with phosphate buffered saline. The cells are re-suspended in PBS buffer and incubated at 37° C. in the dark for 15 minutes with 250 nM TMRM (a cationic dye which accumulates within mitochondria in accordance with the ΔψmNernst potential). Cells are collected by

centrifugation at 300×g for 3 minutes and then washed twice with phosphate buffered saline. The samples are analyzed immediately by flow cytometry using 488 nm excitation laser and the FL2-H channel. The protonophore FCCP (30 μM) will be used to dissipate the chemiosmotic proton gradient ($\Delta\mu H^+$) and serves as a control for loss of $\Delta\psi m$. The results obtained will be verified in three independent experiments.

[0755] *Trypan Blue Cell Viability Assay*: This technique is used to assess the cytoprotective effects of mitochondrial-targeted creatine compounds in cultured cells pharmacologically treated to induce cell death by GSH depletion. DEM is used to deplete cellular GSH and induce oxidative stress. The viability of DEM-treated cells is determined by their ability to exclude the dye trypan blue. Viable cells exclude trypan blue; whereas, non-viable cells take up the dye and stain blue. Briefly, cells are seeded at a density of 1×10^6 cells/mL and treated with different concentrations of mitochondrial-targeted creatine compounds. Cells are incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for three hours with 5 mM DEM. Cell viability is determined by staining cells with 0.4% trypan blue using a hemocytometer. At least 500 cells are counted for each experimental group.

[0756] *Cytochrome c Reduction Assay*: The rate of cytochrome c (10 μM) reduction is measured by monitoring the change in absorbance at 550 nm. Briefly the reaction is initiated by addition of 100 μM of a mitochondrial-targeted creatine compound to a mixture containing 50 mM phosphate buffer, 0.1 mM EDTA, pH 7.8, and 10 μM cytochrome c (Sigma, St. Louis, Mo. USA). For cytochrome c reduction by superoxide, xanthine oxidase (0.01 IU/mL) (Sigma, St. Louis, Mo. USA) is used in presence of xanthine (50 μM).

[0757] *Total Cellular ATP Concentration Assay*: The reductions of mitochondrial respiratory chain activity in CoQ10 deficient patients have been reported (Quinzii G *et al.*, *FASEB J.* 22:1874-1885 (2008)). Briefly, lymphocytes (2×10^5 cell/mL), are plated (1 mL in 12-well plates) and treated with mitochondrial-targeted creatine compounds at final concentrations of 5, 10 μM, and 25 μM and incubated at 37° C for 48 hours in a humidified atmosphere containing 5% CO_2 in air. Mitochondrial-targeted creatine compounds are prepared by first making 20 mM stock solutions in DMSO. Cells are transferred (100 μL) to 96-well microtiter black-walled cell culture plates (Costar, Corning, N.Y.). The total intracellular ATP level is measured in a luminator (Clarity™ luminescence microplate reader) with the ATP Bioluminescence Assay Kit (ViaLight® Plus ATP monitoring reagent kit, Lonza) following the manufacturer's instructions. The standard curve of ATP is obtained

by serial dilution of 1 mM ATP solution. After calibration against the ATP standard, the ATP content of the cell extract is determined and normalized for protein content in the cell.

[0758] *Mitochondrial Bioenergetics Assessment*: The use of mitochondrial-targeted creatine compounds and methylene blue analogues (positive control) to normalize and restore the respiratory chain activities in cultured cells derived from subjects with a mitochondrial disease or disorder are assessed. Lymphocytes are cultured under glucose-free media supplemented with galactose for two weeks to force energy production predominantly through oxidative phosphorylation rather than glycolysis. Lymphocytes are cultured in RPMI 1640 medium glucose-free supplemented with 25 mM galactose, 2 mM glutamine and 1% penicillin-streptomycin, and 10%, dialyzed fetal bovine serum FBS (<0.5 μg/mL). Briefly, lymphocytes (2×10^5 cell/mL), are plated (1 mL in 12-well plates) and treated with the mitochondrial-targeted creatine compounds at final concentrations of 50, 125, 250, 1000, and 5000 nM, and incubated at 37° C for 48 hours in a humidified atmosphere containing 5% CO_2 in air. Cells are transferred (100 μL) to 96-well microtiter black-walled cell culture plates. The total intracellular ATP level is measured in a luminator (Clarity™ luminescence microplate reader) with the ATP Bioluminescence Assay Kit (ViaLight®-Plus ATP monitoring reagent kit, Lonza) following the manufacturer's instructions. Carbonyl cyanide-p-trifluormethoxy-phenylhydrazone (FCCP) and oligomycin are used as controls for inhibition of ATP synthesis.

[0759] *Results* – It is anticipated that cells derived from subjects suspected of having or diagnosed as having a mitochondrial disease or disorder will show one or more alterations associated with mitochondrial dysfunction such as decreased cell viability, loss of mitochondrial membrane potential, decreased cytochrome c reduction, decreased cellular content of ATP, and reduced efficiency of oxidative phosphorylation. It is expected that treatment with mitochondrial-targeted creatine compounds will reduce the severity or eliminate one or more of these alterations associated with mitochondrial dysfunction.

[0760] These results will show that the mitochondrial-targeted creatine compounds of the present technology are useful in methods for normalizing and restoring mitochondrial bioenergetics generally.

Example 20 – Use of Mitochondrial-Targeted Creatine Compounds in Combination with an Additional Therapeutic Agent in Ameliorating Symptoms of a Mitochondrial Disease or Disorder in Subjects Diagnosed with a Mitochondrial Disease or Disorder

[0761] This Example will demonstrate the use of mitochondrial-targeted creatine compounds of the present technology, in combination with one or more additional therapeutic agents (*e.g.*, one or more of vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents) to alleviate or ameliorate one or more symptoms in a subject diagnosed with a mitochondrial disease. Suitable test subjects diagnosed with a mitochondrial disease will exhibit one or more of the following symptoms: poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, and brain atrophy.

[0762] Subjects diagnosed with any mitochondrial disease or disorder described herein and exhibiting one or more of the above symptoms are divided into 4 groups (N=20) as follows: Group I is administered a daily dose of 0.5 mg/kg/day of a mitochondrial-targeted creatine compound; Group II is administered a daily dose of between 0.01-10 mg/k/day of one or more of vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents; Group III is administered a combination of a daily dose of 0.5 mg/kg/day of the mitochondrial-targeted creatine compound and a daily dose of between 0.01-10 mg/k/day of one or more of vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents; and Group IV is administered saline vehicle (control). Each group will receive therapy for six weeks. At the end of the six-week test period, subjects are evaluated for amelioration or attenuation of one or more of the symptoms described above.

[0763] It is anticipated that subjects in groups I and II will show an improvement (*e.g.*, alleviation, amelioration) in at least one or more of the signs and symptoms of the mitochondrial disease or disorder as compared to the control group, Group IV. It is anticipated that subjects in Group III will exhibit a synergistic effect with respect to the combination therapy, and will exhibit a greater improvement in one or more signs or symptoms of the mitochondrial disease or disorder than the subjects of Group I and II.

[0764] These results will show that combination therapy, *i.e.*, mitochondrial-targeted creatine compounds of the present technology in combination with one or more additional therapeutic agents, is useful in reducing, alleviating or ameliorating one or more of the signs and symptoms associated with a mitochondrial disease or disorder.

EQUIVALENTS

[0765] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of the present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. The present technology is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. It is to be understood that the present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0766] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0767] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down

into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[0768] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

[0769] Other embodiments are set forth within the following claims.

CLAIMS

What is claimed is:

1. A method for treating or preventing a mitochondrial disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a mitochondrial-targeted creatine compound of Formula II or Formula III, in combination with one or more additional therapeutic agents selected from the group consisting of: vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents.

2. The method of claim 1, wherein the mitochondrial disease or disorder is selected from the group consisting of Alexander disease, Alpers Syndrome, Alpha-ketoglutarate dehydrogenase (AKDGH) deficiency, ALS-FTD, Sideroblastic anemia with spinocerebellar ataxia, Pyridoxine-refractory sideroblastic anemia, GRACILE Syndrome, Björnstad Syndrome, Leigh Syndrome, mitochondrial complex III deficiency nuclear type 1 (MC3DN1), combined oxidative phosphorylation deficiency 18 (COXPD18), Thiamine-responsive megaloblastic anemia syndrome (TRMA), Pearson Syndrome, HAM Syndrome, Ataxia, Cataract, and Diabetes Syndrome, MELAS/MERRF Overlap Syndrome, combined oxidative phosphorylation deficiency-14 (COXPD14), Infantile cerebellar-retinal degeneration (ICRD), Charlevoix-Saguenay spastic ataxia, Primary coenzyme Q10 deficiency-1 (COQ10D1), ataxia oculomotor apraxia type 1 (AOA1), Autosomal recessive spinocerebellar ataxia-9/ coenzyme Q10 deficiency-4 (COQ10D4), Ataxia, Pyramidal Syndrome, and Cytochrome Oxidase Deficiency, Friedreich's ataxia, Infantile onset spinocerebellar ataxia (IOSCA)/ Mitochondrial DNA Depletion Syndrome-7, Leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), Autosomal recessive spastic ataxia-3 (SPAX3), MIRAS, SANDO, mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), spastic ataxia with optic atrophy (SPAX4), progressive external ophthalmoplegia with mitochondrial DNA deletions autosomal dominant type 5 (PEOA5), mitochondrial complex III deficiency nuclear type 2 (MC3DN2), episodic encephalopathy due to thiamine pyrophosphokinase deficiency/Thiamine Metabolism Dysfunction Syndrome-5 (THMD5), Spinocerebellar ataxia-28 (SCA28), autosomal dominant cerebellar ataxia,

deafness, and narcolepsy (ADCA-DN), Dominant Optic Atrophy (DOA), cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS) Syndrome, spinocerebellar ataxia 7 (SCA7), Barth Syndrome, Biotinidase deficiency, gyrate atrophy, Syndromic Dominant Optic Atrophy and Deafness (Syndromic DOAD), Dominant Optic Atrophy plus (DOAplus), Leber's hereditary optic neuropathy (LHON), Wolfram Syndrome-1 (WFS1), Wolfram Syndrome-2 (WFS2), Age-related macular degeneration (ARMD), Brunner Syndrome, Left ventricular noncompaction-1 (LVNC1), histiocytoid cardiomyopathy, Familial Myalgia Syndrome, Parkinsonism, Fatal infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency-1 (CEMCOX1), Sengers Syndrome, Cardiofaciocutaneous Syndrome-1 (CFC1), Mitochondrial trifunctional protein (MTP) deficiency, infantile encephalocardiomyopathy with cytochrome c oxidase deficiency, cardiomyopathy + encephalomyopathy, mitochondrial phosphate carrier deficiency, infantile cardioencephalomyopathy due to cytochrome c oxidase (COX) deficiency (CEMCOX2), β-Hydroxyisobutyryl CoA Deacylase (HIBCH) deficiency, ECHS1) deficiency, Maternal Inheritance Leigh Syndrome (MILS), dilated cardiomyopathy with ataxia (DCMA), Mitochondrial DNA Depletion Syndrome-12 (MTDPS12), cardiomyopathy due to mitochondrial tRNA deficiencies, mitochondrial complex V (ATP synthase) deficiency nuclear type 1 (MC5DN1), combined oxidative phosphorylation deficiency-8 (COXPD8), progressive leukoencephalopathy with ovarian failure (LKENP), combined oxidative phosphorylation deficiency-10 (COXPD10), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-17 (COXPD17), combined oxidative phosphorylation deficiency-5 (COXPD5), combined oxidative phosphorylation deficiency-9 (COXPD9), carnitine acetyltransferase (CRAT) deficiency, carnitine palmitoyltransferase I (CPT I) deficiency, myopathic carnitine deficiency, primary systemic carnitine deficiency (CDSP), carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acylcarnitine translocase deficiency (CACTD), cartilage-hair hypoplasia, cerebrotendinous xanthomatosis (CTX), congenital adrenal hyperplasia (CAH), megaconial type congenital muscular dystrophy, cerebral creatine deficiency syndrome-3 (CCDS3), maternal nonsyndromic deafness, maternal nonsyndromic deafness, autosomal dominant deafness-64 (DFNA64), Mohr-Tranebjaerg Syndrome, Jensen Syndrome, MEGDEL, reticular dysgenesis, primary coenzyme Q10 deficiency-6 (COQ10D6), CAGSSS, diabetes, Dimethylglycine dehydrogenase

deficiency (DMGDHD), Multiple Mitochondrial Dysfunctions Syndrome-1 (MMDS1), Multiple Mitochondrial Dysfunctions Syndrome-2 (MMDS2), Multiple Mitochondrial Dysfunctions Syndrome-3 (MMDS3), childhood leukoencephalopathy associated with mitochondrial Complex II deficiency, encephalopathies associated with mitochondrial Complex I deficiency, encephalopathies associated with mitochondrial Complex III deficiency, encephalopathies associated with mitochondrial Complex IV deficiency, encephalopathies associated with mitochondrial Complex V deficiency, hyperammonemia due to carbonic anhydrase VA deficiency (CA5AD), early infantile epileptic encephalopathy-3 (EIEE3), 2,4-Dienoyl-CoA reductase deficiency (DECRD), infection-induced acute encephalopathy-3 (IIAE3), ethylmalonic encephalopathy (EE), hypomyelinating leukodystrophy (HLD4), exocrine pancreatic insufficiency, dyserythropoietic anemia and calvarial hyperostosis, Glutaric aciduria type 1 (GA-1), glycine encephalopathy (GCE), hepatic failure, 2-hydroxyglutaric aciduria, 3-hydroxyacyl-CoA dehydrogenase deficiency, familial hyperinsulinemic hypoglycemia (FHH), hypercalcemia infantile, hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) Syndrome, Immunodeficiency with hyper-IgM type 5 (HIGM5), Inclusion Body Myositis (IBM), polymyositis with mitochondrial pathology, IM-Mito, granulomatous myopathies with anti-mitochondrial antibodies, necrotizing myopathy with pipestem capillaries, myopathy with deficient chondroitin sulfate C in skeletal muscle connective tissue, benign acute childhood myositis, idiopathic orbital myositis, masticator myopathy, hemophagocytic lymphohistiocytosis, infection-associated myositis, Facioscapulohumeral dystrophy (FSH), familial idiopathic inflammatory myopathy, Schmidt Syndrome (Diabetes mellitus, Addison disease, Myxedema), TNF receptor-associated Periodic Syndrome (TRAPS), focal myositis, autoimmune fasciitis, Spanish toxic oil-associated fasciitis, Eosinophilic fasciitis, Macrophagic myofasciitis, Graft-vs-host disease fasciitis, Eosinophilia-myalgia Syndrome, perimyositis, isovaleric acidemia (IVA), Kearnes-Sayre Syndrome (KSS), 2-oxoadipic aciduria, 2-aminoadipic aciduria, Limb-girdle Muscular Dystrophy Syndromes, leukodystrophy, Maple syrup urine disease (MSUD), 3-Methylcrotonyl-CoA carboxylase (MCC), Methylmalonic aciduria (MMA), Miller Syndrome, Mitochondrial DNA Depletion Syndrome-2 (MTDPS2), spinal muscular atrophy syndrome, rigid spine syndrome, severe myopathy with motor regression, Mitochondrial DNA Depletion Syndrome-3, MELAS Syndrome, camptocormia,

MNGIE, MNGIM Syndrome, Menkes Disease, Occipital Horn Syndrome, X-linked distal spinal muscular atrophy-3 (SMAX3), methemoglobinemia, MERRF, progressive external ophthalmoplegia with myoclonus, deafness and diabetes (DD), multiple symmetric lipomatosis, Myopathy with Episodic high Creatine Kinase (MIMECK), Epilepsia Partialis Continua, malignant hyperthermia syndromes, glycogen metabolic disorders, fatty acid oxidation and lipid metabolism disorders, medication-, drug- or toxin-induced myoglobinuria, mitochondrial disorder-associated myoglobinuria, hypokalemic myopathy and rhabdomyolysis, muscle trauma-associated myoglobinuria, ischemia-induced myoglobinuria, infection-induced myoglobinuria, immune myopathies associated with myoglobinuria, Myopathy, lactic acidosis, and sideroblastic anemia (MLASA), infantile mitochondrial myopathy due to reversible COX deficiency (MMIT), Myopathy, Exercise intolerance, Encephalopathy and Lactic acidemia Syndrome, myoglobinuria and exercise intolerance syndrome, exercise intolerance, proximal weakness ± myoglobinuria syndrome, encephalopathy and seizures syndrome, septo-optic dysplasia, exercise intolerance ± mild weakness, myopathy ± exercise intolerance, growth or CNS disorder, maternally-inherited mitochondrial myopathies, myopathy with lactic acidosis, myopathy with rhabdomyolysis, Myopathy with cataract and combined respiratory chain deficiency, myopathy with abnormal mitochondrial translation, Fatigue Syndrome, myopathy with extrapyramidal movement disorders (MPXPS), glutaric aciduria II (MADD), primary CoQ10 deficiency-1 (COQ10D1), primary CoQ10 deficiency-2 (COQ10D2), primary CoQ10 deficiency-3 (COQ10D3), primary CoQ10 deficiency-5 (COQ10D5), secondary CoQ10 deficiency, autosomal dominant mitochondrial myopathy, myopathy with focal depletion of mitochondria, mitochondrial DNA breakage syndrome (PEO + Myopathy), lipid type mitochondrial myopathy, multiple symmetric lipomatosis (MSL), N-acetylglutamate synthase (NAGS) deficiency, Nephronophthisis (NPHP), ornithine transcarbamylase (OTC) deficiency, neoplasms, NARP Syndrome, paroxysmal nonkinesigenic dyskinesia (PNKD), sporadic PEO, maternally-inherited PEO, autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-3 (PEOA3), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-2 (PEOA2), autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions-1 (PEOA1), PEO+ demyelinating neuropathy, PEO + hypogonadism, autosomal dominant progressive external ophthalmoplegia with

mitochondrial DNA deletions-4 (PEOA4), distal myopathy, cachexia & PEO, autosomal dominant progressive external ophthalmoplegia-6 (PEOA6), PEO + Myopathy and Parkinsonism, autosomal recessive progressive external ophthalmoplegia (PEOB), Mitochondrial DNA Depletion Syndrome-11 (MTDPS11), PEO with cardiomyopathy, PEPCK deficiency, Perrault Syndromes (PRLTS), propionic acidemia (PA), pyruvate carboxylase deficiency, pyruvate dehydrogenase E1-alpha deficiency (PDHAD), pyruvate dehydrogenase E1-beta deficiency (PDHBD), dihydrolipoamide dehydrogenase (DLD) deficiency, pyruvate dehydrogenase phosphatase deficiency, pyruvate dehydrogenase E3-binding protein deficiency (PDHXD), mitochondrial pyruvate carrier deficiency (MPYCD), Schwartz-Jampel Syndrome type 1 (SJS1), selenium deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency, succinyl CoA:3-oxacid CoA transferase (SCOT) deficiency, Stuve-Wiedemann Syndrome (STWS), thrombocytopenia (THC), Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD), Vitamin D-dependent rickets type 1A (VDDR1A), Wilson's disease, Zellweger Syndrome (PBD3A), arsenic trioxide myopathy, myopathy and neuropathy resulting from nucleoside analogues, germanium myopathy, Parkinsonism and mitochondrial Complex I neurotoxicity due to trichloroethylene, valproate-induced hepatic failure, neurodegeneration with brain iron accumulation-4 (NBIA4), Complex I deficiency, Complex II deficiency, Complex III deficiency, Complex IV deficiency, Complex V deficiency, Cytochrome c oxidase (COX) deficiency, combined complex I, II, IV, ± V deficiency, combined complex I, II, and III deficiency, combined oxidative phosphorylation deficiency-1 (COXPD1), combined oxidative phosphorylation deficiency-2 (COXPD2), combined oxidative phosphorylation deficiency-3 (COXPD3), combined oxidative phosphorylation deficiency-4 (COXPD4), combined oxidative phosphorylation deficiency-6 (COXPD6), combined oxidative phosphorylation deficiency-7 (COXPD7), combined oxidative phosphorylation deficiency-9 (COXPD9), combined oxidative phosphorylation deficiency-11 (COXPD11), combined oxidative phosphorylation deficiency-12 (COXPD12), combined oxidative phosphorylation deficiency-13 (COXPD13), combined oxidative phosphorylation deficiency-15 (COXPD15), combined oxidative phosphorylation deficiency-16 (COXPD16), combined oxidative phosphorylation deficiency-19 (COXPD19), combined oxidative phosphorylation deficiency-20 (COXPD20), combined oxidative phosphorylation deficiency-21 (COXPD21), fumarase deficiency,

HMG-CoA synthase-2 deficiency, hyperuricemia, pulmonary hypertension, renal failure, and alkalosis (HUPRA) Syndrome, syndromic microphthalmia-7, pontocerebellar hypoplasia type 6 (PCH6), Mitochondrial DNA Depletion Syndrome-9 (MTDPS9P), and Sudden infant death Syndrome (SIDS).

3. The method of any one of claims 1-2, wherein the mitochondrial-targeted creatine compound is administered daily for one, two, three, four or five weeks.

4. The method of any one of claims 1-3, wherein the mitochondrial-targeted creatine compound is administered daily for 6 weeks or more.

5. The method of claim 1, wherein the subject displays abnormal levels of one or more energy biomarkers compared to a normal control subject.

6. The method of claim 5, wherein the energy biomarker is selected from the group consisting of lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; lactate/pyruvate ratios; total, reduced or oxidized glutathione levels; total, reduced or oxidized cysteine levels; reduced/oxidized glutathione ratios; reduced/oxidized cysteine ratios; phosphocreatine levels; NADH (NADH+H^{30}) or NADPH (NADPH+H^{30}) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; oxidized cytochrome C/reduced cytochrome C ratio; acetoacetate levels; beta-hydroxy butyrate levels; acetoacetate/ beta-hydroxy butyrate ratio; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; levels of reactive oxygen species; oxygen consumption (VO2), carbon dioxide output (VCO2), and respiratory quotient (VCO2/VO2).

7. The method of any one of claims 1-6, wherein the subject is human.

8. The method of any one of claims 1-7, wherein the mitochondrial-targeted creatine compound is administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly.

9. The method of claim 1, wherein the symptoms of the mitochondrial disease or disorder comprises one or more of poor growth, loss of muscle coordination, muscle

weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, and brain atrophy.

10. The method of any one of claims 1-8, wherein the mitochondrial-targeted creatine compound and the one or more additional therapeutic agents are administered separately, sequentially, or simultaneously to the subject.

11. A method for modulating the expression of one or more energy biomarkers in a mammalian subject in need thereof, the method comprising: administering to the subject a therapeutically effective amount of a mitochondrial-targeted creatine compound of Formula II or Formula III, in combination with one or more additional therapeutic agents selected from the group consisting of: vitamins, cofactors, antibiotics, hormones, antineoplastic agents, steroids, immunomodulators, dermatologic drugs, antithrombotic, antianemic, and cardiovascular agents.

12. The method of claim 11, wherein the energy biomarker is selected from the group consisting of lactic acid (lactate) levels; pyruvic acid (pyruvate) levels; lactate/pyruvate ratios; total, reduced or oxidized glutathione levels; total, reduced or oxidized cysteine levels; reduced/oxidized glutathione ratios; reduced/oxidized cysteine ratios; phosphocreatine levels; NADH ($NADH+H^{30}$) or NADPH ($NADPH+H^{30}$) levels; NAD or NADP levels; ATP levels; reduced coenzyme Q (CoQred) levels; oxidized coenzyme Q (CoQox) levels; total coenzyme Q (CoQtot) levels; oxidized cytochrome C levels; reduced cytochrome C levels; oxidized cytochrome C/reduced cytochrome C ratio; acetoacetate levels; beta-hydroxy butyrate levels; acetoacetate/ beta-hydroxy butyrate ratio; 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels; levels of reactive oxygen species; oxygen consumption (VO2), carbon dioxide output (VCO2), and respiratory quotient (VCO2/VO2).

13. The method of any one of claims 11-12, wherein the mitochondrial-targeted creatine compound is administered daily for one, two, three, four, or five weeks.

14. The method of any one of claims 11-12, wherein the mitochondrial-targeted creatine compound is administered daily for 6 weeks or more.

15. The method of any one of claims 11-14, wherein the subject has been diagnosed as having, is suspected of having, or is at risk of having a mitochondrial disease or disorder.

16. The method of claim 15, wherein symptoms of the mitochondrial disease or disorder comprises one or more of poor growth, loss of muscle coordination, muscle weakness, neurological deficit, seizures, autism, autistic spectrum, autistic-like features, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, severe constipation, diabetes, increased risk of infection, thyroid dysfunction, adrenal dysfunction, autonomic dysfunction, confusion, disorientation, memory loss, failure to thrive, poor coordination, sensory (vision, hearing) problems, reduced mental functions, hypotonia, disease of the organ, dementia, respiratory problems, hypoglycemia, apnea, lactic acidosis, seizures, swallowing difficulties, developmental delays, movement disorders (dystonia, muscle spasms, tremors, chorea), stroke, and brain atrophy.

17. The method of any one of claims 11-16, wherein the subject is human.

18. The method of any one of claims 11-17, wherein the mitochondrial-targeted creatine compound is administered orally, intranasally, intrathecally, intraocularly, intradermally, transmucosally, iontophoretically, topically, systemically, intravenously, subcutaneously, intraperitoneally, or intramuscularly.

19. The method of any one of claims 11-18, wherein the mitochondrial-targeted creatine compound and the one or more additional therapeutic agents are administered separately, sequentially, or simultaneously to the subject.

FIG. 1

Site of Dysfunction	Biochemical Event	Measurable Energy Biomarker	Physical Effect
Respiratory Chain	↑ NADH	Δ lactate, Δ lactate:pyruvate ratio; and Δ acetoacetate:β-hydroxy butyrate ratio	Metabolic dyscrasia & fatigue
Respiratory Chain	↓ H^+ gradient	Δ ATP	Organ dependent dysfunction
Respiratory Chain	↓ Electron flux	Δ VO_2, RQ, BMR, ΔT, AT, pH	Metabolic dyscrasia & fatigue
Mitochondria & cytosol	↓ ATP, ↓ VO_2	Δ Work, ΔHR (CO)	Exercise intolerance
Mitochondria & cytosol	↓ ATP	Δ PCr	Exercise intolerance
Respiratory Chain	↓ Cyt $C_{Ox/Red}$	Δλ ~700–900 nM (Near Infrared Spectroscopy)	Exercise intolerance
Intermediary metabolism	↓ Catabolism	Δ C^{14}-Labeled substrates	Metabolic dyscrasia & fatigue
Respiratory Chain	↓ Electron flux	Δ Mixed Venous VO_2	Metabolic dyscrasia & fatigue
Mitochondria & cytosol	↑ Oxidative stress	Δ Tocopherol & Tocotrienols, CoQ10, docosahexanoic acid	Uncertain
Mitochondria & cytosol	↑ Oxidative stress	Δ $Glutathione_{red}$	Uncertain
Mitochondria & cytosol	Nucleic acid oxidation	Δ8-hydroxy 2-deoxy guanosine	Uncertain
Mitochondria & cytosol	Lipid oxidation	ΔIsoprostane(s), eicasanoids	Uncertain
Cell membranes	Lipid oxidation	ΔEthane (breath)	Uncertain
Cell membranes	Lipid oxidation	ΔMalondialdehyde	Uncertain