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(54) **DIAGNOSIS OF LYSOSOMAL STORAGE DISORDERS USING SAPOSINS AND OTHER MARKERS**

DIAGNOSE VON LYSOSOMALEN SPEICHERUNGSKRANKHEITEN UNTER VERWENDUNG VON SAPOSINEN UND ANDEREN MARKERN

DIAGNOSTIC DE TROUBLES LYSOSOMIAUX A L'AIDE DE SAPOSINES ET D'AUTRES MARQUEURS

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## Description

### Background

**[0001]** Lysosomal storage disorder (LSDs) are a large family of genetic disorders that can lead to the manifestation of severe clinical symptoms. Although the frequency of each individual disease is relatively rare, the collective incidence of all lysosomal storage disorders is about 1 in 7000 newborns. This is greater than that of other diseases, such as phenylketonuria, for which newborn screening methods are available. Each lysosomal storage disorder results from a deficiency in a lysosomal enzyme, transporter or protein involved in lysosomal biogenesis or function [1]. The deficiency leads to the accumulation of substrates, normally degraded within the lysosome, and an increase in size and number of lysosomes within the cells.

**[0002]** Predominantly affecting young children, LSDs can be very severe with a wide range of clinical symptoms that depend on the particular genotype involved including mental retardation, skeletal abnormalities, organomegaly, corneal clouding and coarse facial features [2] [1]. In recent years treatments for several LSDs have become possible including drug therapy [3-5], bone marrow transplantation [2, 6], and enzyme replacement therapy [6, 7]. New treatment protocols are being developed with the increased knowledge of the underlying cause of the specific disorders, and the availability of animal models together with the development of new technologies. Animal models are of particular importance for the testing of new treatments such as enzyme replacement therapy [2, 6, 8, 9], and studies in animal models have also shown that maximum efficacy, in most cases, is achieved when treatment is given at an early stage of pathology [10, 11].

**[0003]** In most of the disorders, clinical pathology is not apparent at birth but presents in the first few years of life. For current and proposed therapies to achieve maximum efficacy it is important that the disorders are detected early, before the onset of irreversible pathology, particularly if there is central nervous system and/or bone pathology involvement. Thus, there is a need for screening methods for newborns, preferably screening methods that can detect all or most lysosomal disorders with a minimum number of assays. The present invention fulfils this and other needs.

### Definitions

**[0004]** A patient is a subject, typically human, either living or subject to postmortem analysis, undergoing a diagnostic test to determine whether the patient has a disorder, susceptibility thereto, or above-normal risk thereof. A patient has a disease if the patient is currently showing symptoms thereof. A patient is susceptible to the disease if the patient is presently asymptomatic but has a genetic or other disposition to acquire the disease at a later time. A patient is at above normal risk of having the disease if the patient possesses a genetic or biochemical marker correlated with presence of the disease in a population of individuals. Some patients undergoing a diagnostic test are suspected of having the disease from symptoms, family history or other tests previously conducted. Other patients undergoing a diagnostic test have no known symptoms, or other risk factor for the disease, but undergo the test as a precautionary screening, optionally in conjunction with other tests.

**[0005]** A diagnostic test serves to indicate that a patient has a disorder being tested for, is susceptible to such a disorder, or has above normal risk of having such a disorder relative to the general population.

**[0006]** Unless the context requires otherwise, the word "comprise," or variations such as "comprises" or "comprising" imply the inclusion of a stated element or integer or group of elements or integers, but not the exclusion of any other element or integer or group of elements or integers.

**[0007]** Specific binding of a reagent with an analyte to form a complex means that the complex has a dissociation constant of less than micromolar.

### Summary of the Claimed Invention

**[0008]** Provided are methods of diagnosing or monitoring a lysosomal storage disease in a patient. The methods entail measuring the level of at least one saposin in a tissue sample of the patient, the level providing an indication of the presence or absence or extent of the disorder in the patient. In some methods, the sample is a plasma sample. In some methods, presence of a disorder is indicated by a measured level of saposin exceeding a mean level of the saposin in a control population of individuals not having a lysosomal storage disease. In some methods, presence of a disorder is indicated when the measured level of saposin exceeds the 95th percentile level in the control population. In some methods, the patient is not known to have a lysosomal storage disorder before the measuring step. In some methods, the patient is less than one year old. In some methods, the patient is a foetus. The saposin detected in such methods can be any of saposin A, B, C or D, prosaposin, mRNA encoding any of these or subtypes of these saposins. In some methods, saposins are detected using an antibody as the diagnostic reagent. In some methods, the lysosomal storage disease is cystinosis, Fabry's disease, Niemann-Pick disease, Pompe's disease or Wolman's disease. Some methods also include a step of informing a patient, a parent or a guardian of the results of the presence of a lysosomal storage

disease indicated by the measuring step.

**[0009]** Provided are methods of diagnosing or monitoring a lysosomal storage disorder in a patient in which the level of α-glucosidase is measured in a tissue sample from a patient. The level of the α glucosidase provides an indicator of the presence or extent of the disorder in the patient. In some methods, the sample is a plasma sample or whole blood sample. In some methods, the measuring step indicates presence of acid lipase disease, mannosidosis, mucopolysaccharidosis II (MPSII), MPS IIIA, MSD, mucolipidosis, N-P (A/B), N-P (C), Sandhoff, SAS or TSD B1 from an increased concentration of α-glucosidase relative to the mean level in a control population of individuals not having a lysosomal storage disease. In some methods, the measuring step indicates presence of MPS IVA or Pompe's disease from a decreased level of α glucosidase relative to the mean level in a control population of individuals not having lysosomal storage disease.

**[0010]** Provided are methods of diagnosing a lysosomal storage disease by measuring a level of a saposin, measuring a level of LAMP-1 or LAMP-2 and measuring a level of α glucosidase in the same or different tissue samples from a patient. Presence of increased levels of saposin, LAMP-1 or LAMP-2 and either increased or decreased levels of α glucosidase relative to corresponding levels in a control population of individuals not having a lysosomal storage disorder is an indicator of a lysosomal storage disorder.

**[0011]** Provided are diagnostic kits. Some such kits comprise a first reagent that binds to a LAMP and a second reagent that binds to a saposin. Some kits further comprise a third reagent that binds to a glucosidase.

**[0012]** Provided are methods of monitoring treatment of a lysosomal storage disease in a patient. Such methods entail determining a baseline level of a saposin in a tissue sample from a patient with a lysosomal storage disorder before treatment with an agent. The baseline level is then compared with a level in a sample obtained after the patient has been treated with the agent. A reduction in the level of saposin after treatment relative to the baseline indicates a positive treatment outcome

**[0013]** Provided are also provides methods of monitoring treatment of acid lipase disease, mannosidosis, MPSII, MPS IIIA, MSD, mucolipidosis, N-P (A/B). N-P(C), Sandhoff, SAS or TSD B1. Such methods entail determining a baseline level of α glucosidase in a tissue sample from the patient with the disorder before treatment with an agent. The baseline level is then compared with a level of the α glucosidase in a tissue sample from the patient with the disorder after treatment with the agent. A decrease relative to the baseline indicates a positive treatment outcome.

**[0014]** Provided are methods of monitoring a patient with galactosialidosis, MPS IVA or Pompe's disease. Such methods entail determining a baseline level of α glucosidase in a tissue sample from the patient with the disorder before treatment with the agent. The baseline level is then compared with a level of the α-glucosidase in a tissue sample from the patient after treatment with the agent. An increase relative to the baseline indicates a positive treatment outcome.

**Brief Description of the Drawings**

**[0015]**

Figure 1. Calibration curves for saposins A, B, C and D immunoquantification assays. Optimum immunoquantification conditions were used to generate saposin A (panel a), saposin B (panel b), saposin C (panel c) and saposin D (panel d) calibration curves for use in the immunoquantification of the respective proteins. Microtiter plates were coated with primary anti-saposin polyclonal antibody (2.5mg/L, 4°C, overnight), standards were incubated (4°C, 4h) and detected with Eu-labelled anti-saposin polyclonal antibody (0.25mg/L, 4°C, overnight).

Figure 2. Box plots of saposin concentrations in plasma from control and LSD-affected individuals. Saposin concentrations were determined by using 0.25-2μL plasma samples. N = number of samples used in each group. The line within the box is the median level, shaded areas below and above the median represent the 25$^{th}$ and 75$^{th}$ centiles, respectively, and bars represent the range. O depicts the outliers, and * denotes extreme outliers. Galact = galactosialidosis; GM I = GM I gangliosidosis; Mann = α-Mannosidosis; MLD = metachromatic leukodystrophy; MSD = Multiple Sulphatase Deficiency; NCL = Neuronal Ceroid Lipofuscinosis; N-P = Niemann-Pick disease; SAS = Sialic Acid Storage disease; TSD = Tay-Sachs disease.

Figure 3 Box plot of α-glucosidase levels in plasma from control and LSD affected individuals Alpha-glucosidase concentrations were determined by using 5.0μL plasma samples. N = number of samples used in each group. The line within the box is the median level, shaded areas below and above the median represent the 25$^{th}$ and 75$^{th}$ centiles, respectively, and bars represent the range. O depicts the outliers, and * denotes extreme outliers. Galact = galactosialidosis; GM I = GM I gangliosidosis; MLD = metachromatic leukodystrophy; MSD = Multiple Sulphatase Deficiency; N-P = Niemann-Pick disease; SAS = Sialic Acid Storage disease; TSD = Tay-Sachs disease. The Y axis has been expanded to highlight differences between the control and LSD patient

## Detailed Description

1. General

**[0016]** Provided are methods of diagnosing patients for presence of lysocomal storage disorders. The methods work by detecting a biological marker correlated with at least one or a subset of such disorders. Some methods detect one or more naturally occurring polypeptides termed saposins. The presence of above normal levels of such polypeptides in a tissue sample from of a patient is correlated with several types of lysosomal disease including cystinosis, Fabry disease, Niemann-Pick disease (types A/B and C), Pompe's disease, Wolman disease, Krabbe disease, metachromatic leukodystrophy, and Tay-Sachs disease. Other methods entail determining the level of a-glucosidase in a tissue sample from a patient. Levels of α-glucosidase are depressed relative to normal levels in Pompe's disease. Levels of α-glucosidase are elevated relative to normal levels in acid lipase disease, mannosidosis, MPS II, MPS IIIA, multiple sulphatase deficiency, mucolipidosis, Niemann-Pick (A/B), Niemann-Pick (C), sialic acid storage disease or Sandhoff's disease, Tay-Sachs disease A/B.

**[0017]** The methods are suitable for large scale screening of recently born infants or foetuses for the presence of lysosomal storage disorders, optionally, in conjunction with additional biochemical and/or genetic markers of other disorders that may reside in newborns. The methods are also suitable for monitoring patients who have previously been diagnosed with a lysosomal storage disease, particularly their response to treatment. Methods of analysing of saposins and α-glucosidase can be performed in combination, optionally in further combination with detecting other biochemical markers correlated with lysosomal storage disorders, as described by WO 97/44668. Optionally, analysis of biochemical markers can also be combined with polymorphic analysis of genes encoding lysosomal enzymes for polymorphisms correlated with disease.

II. Lysosomal Storage Disorders

**[0018]** There are over thirty lysosomal diseases, each resulting from a deficiency of a particular lysosomal protein, usually as a result of genetic mutation. See, eg., Cotran et al., Robbins Pathologic Basis of Disease (4th ed. 1989) (incorporated by reference in its entirety for all purposes). The deficiency in the lysosomal protein usually results in harmful accumulation of a metabolite. For example, in Hurler's, Hunter's, Morquio's, and Sanfilippo's syndromes, there is an accumulation of mucopolysaccharides; in Tay-Sachs, Gaucher, Krabbe, Niemann-Pick, and Fabry syndromes, there is an accumulation of sphingolipids; and in fucosidosis and mannosidosis, there is an accumulation of fucose-containing sphingolipids and glycoprotein fragments, and of mannose-containing oligosaccharides, respectively.

**[0019]** Glycogen storage disease type II (GSD II; Pompe's disease; acid maltase deficiency) is caused by deficiency of the lysosomal enzyme acid α-glucosidase (acid maltase). Three clinical forms are distinguished: infantile, juvenile and adult. Infantile GSD II has its onset shortly after birth and presents with progressive muscular weakness and cardiac failure. This clinical variant is fatal within the first two years of life. Symptoms in adult and juvenile patients occur later in life, and only skeletal muscles are involved. The patients eventually die due to respiratory insufficiency. Patients may exceptionally survive for more than six decades. There is a good correlation between the severity of the disease and the residual acid α-glucosidase activity, the activity being 10-20% of normal in late onset and less than 2% in early onset forms of the disease (see Hirschhorn, The Metabolic and Molecular Bases of Inherited Disease (Schriver et al., eds., 7th ed., McGraw-Hill, 1995), pp. 2443-2464).

**[0020]** There are more than 30 known genes encoding lysosomal enzymes including α-glucosidase, α L-iduronidase, iduronate-sulfate sulfatase, hexosaminidase A and B, ganglioside activator protein, arylsulfatase A and B, iduronate sulfatase, heparin N-sulfatase, galacto-ceramidase, α-galactosylceramidase A, sphingomyelinase, α-fucosidase, α-mannosidase, aspartylglycosamine amide hydrolase, acid lipase, N-acetyl- α-D-glucosamine-6-sulfate sulfatase, α-and β-galactosidase, β-glucuronidase, β-mannosidase, ceramidase, galactocerebrosidase, α-N-acetylgalactosaminidase, and protective protein and others. DNA clones containing the genomic or cDNA sequences of many of the known genes encoding lysosomal proteins are available. (Scott et al., Am. J. Hum. Genet. 47, 802-807 (1990); Wilson et al., PNAS 87, 8531-8535 (1990); Stein et al., J. Biol. Chem. 264, 1252-1259 (1989); Ginns et al., Biochem. Biophys. Res. Comm. 123, 574-580 (1984); Hoefsloot et al., EMBO J. 7, 1697-1704 (1988); Hoefsloot et al., Biochem. J. 272, 473-479 (1990); Meyerowitz & Proia, PNAS 81, 5394-5398 (1984); Scriver *et al.,* supra, part 12, pages 2427-2882 and references cited therein)) Other examples of genomic and cDNA sequences are available from GenBank.

### III. Saposins

**[0021]** Saposins are small, heat-stable, glycoproteins of which there are four, termed saposins A, B, C and D. All four saposins are derived from a single 73kD precursor protein called prosaposin. The mature saposins have specific roles in activating and enhancing the activities of their respective lysosomal hydrolases [15, 16]. Saposins are critical in the

control of the glycosphingolipid flux through the lysosomal hydrolytic pathway [15] and genetic defects in sphingolipid hydrolases and/or saposins have been associated with the storage of sphingolipids. [17]. All saposins contain about 80 amino acids and show a high degree of sequence identity to each other. Each saposin contains six cysteine residues in nearly identical positions, all of which form three disulfide bridges providing hairpin type structures. Each saposin also contains N-glycoside type carbohydrate chains. They are linked to identical asparagine residue situated at the 21st position from the N-terminal end of each saposins. Among the four saposins, only saposin A has an additional carbohydrate chain linked to the 42 asparagine residue. Structurally important proline residues are also conserved at nearly identical positions. In all, 13 amino acids are conserved in identical positions in each saposin domain. In addition, there are many amino acids of similar nature, especially hydrophobic amino acid residues, at the same position in each domain. Despite such similarity, they are immunologically distinguishable and functionally different. The saposins are well conserved among different animal species, including human, bovine, rat, pig, and even chicken.

**[0022]** Saposins are required for enzymic hydrolysis of certain sphingolipids by specific lysosomal hydrolases. Although saposins are highly homologous to each other, they show unique and distinct specificities. The first discovered saposin, saposin B, stimulates the hydrolysis reactions by arylsulfatase A, GM1 ganglioside β-galactosidase, and α-galactosidase A. Saposin C also catalyses this reaction, although it has less activity. Saposin D stimulates activities of sphingomyelinase and ceramidase. There are probably three distinct pools of prosaposin in human tissues: one for the lysosomal target, one for secretion, and one for targeting to plasma membrane.

**[0023]** As described in Example 1, elevated levels of saposins correlate positively with presence of several lysosomal storage disorders. Although any of the saposins, and the mRNA encoding the same, can be used as screening marker, saposins A, C and/or D are preferred because these saposins show the strongest correlations. Any or all of at least three mechanisms may explain the observed accumulation of saposins in tissue samples from LSD patients described in Example 1. First, the synthesis of saposins may be stimulated by the accumulation of either a defective enzyme or lipids as a compensatory mechanism. Second, saposins may be co-deposited with substrates that accumulate through lack of processing by a defective enzyme. Third, saposins may fail to be degraded through inability to interact with a nonfunctional lysosomal enzyme. The present invention is not, however, dependent on an understanding of mechanism.

### IV. α-glucosidase

**[0024]** Acid α-glucosidase is the enzyme associated with the lysosomal storage disorder termed Pompe's disease. Alpha-glucosidase is initially synthesised in a precursor form of about 100-110kD. (The apparent molecular weight or relative mobility of the precursor may vary somewhat depending on the method of analysis used, but will typically be within the range 95kD and 120kD.) The proteolytic processing of acid α-glucosidase is complex and involves a series of steps in addition to cleavage of the signal peptide taking place at various subpleural locations. Polypeptides are cleaved off at both the N and C terminal ends, whereby the specific catalytic activity is increased. The main species recognised are a 1101100kD precursor, a 95kD intermediate and 76kD and 70kD mature forms. (Hasidic et al., J. Biol. Chem. 255, 4937-4945 (1980); Ode Elferink et al., Eur. J. Biochem. 139, 489-495 (1984); Reuser et al., J. Biol. Chem. 260, 8336-8341 (1985); Hoefsloot et al., EMBO J. 7, 1697-1704 (1988)).

**[0025]** The present invention provides evidence that levels of acid α-glucosidase are depressed in Pompe's disease and in certain other lysosomal storage disorders. Conversely, levels of acid α-glucosidase are elevated in certain other lysosomal storage disorders including mannosidosis, MPS II, MPS IIIA, MSD, mucolipidosis, Nieman Pick (A/B). Nieman Pick (C), Sandhoff, SAS or Tay Sachs disease A/B. Therefore, acid α-glucosidase levels are a useful biochemical marker for screening for lysosomal storage disorders. The present methods detect each of the various molecular weight forms of acid α glucosidase, combinations thereof, and mRNA encoding acid α-glucosidase.

### V. Other Biochemical Markers

**[0026]** WO 97/44668 describes a number of protein markers, including LAMP-1 and LAMP-2, that are correlated with lysosomal storage diseases: LAMP-1 and LAMP-2 are lysosomal membrane glycoproteins (Dahlgren et al., Biochem. J. 311, 667-674 (1995). LAMP-1 is a preferred marker. Elevated levels of LAMP-1 correlate with Gaucher's disease, galactosialidosis, GM1-gangliosidosis, I-cell disease, acid mannosidosis, MPS I, MPS II, MPS IIIA, MPS IIIB, MPS IIID, MPS IV A, MPS VI, multiple sulphatase deficiency, Sandhoff disease, sialic acid storage disease, and Tay-Sachs (AB) disease (13). Levels of LAMP-2 are also increased in many of the same LSDs as LAMP-1 [14] Several of the LSD's that are not correlated with LAMP-1 are correlated with saposin levels (See Table 1). Thus, combined screens for LAMP-1 and saposins identify more patients with lysosomal storage disorders than either screen alone.

### VI. Tissue Samples

**[0027]** Samples for analysis can be obtained from any organ, tissue, fluid or other biological sample comprising

lysosomes or their component proteins. A preferred tissue sample is whole blood and products derived therefrom, such as plasma and serum. Blood samples can be obtained from blood-spot taken from, for example, a Guthrie card. Other sources of tissue samples are skin, hair, urine, saliva, semen, faeces, sweat, milk, amniotic fluid, liver, heart, muscle, kidney and other body organs. Others sources of tissue are cell lines propagated from primary cells from a patient. Tissue samples are typically lysed to release the protein and/or nucleic acid content of cells within the samples. The protein or nucleic acid fraction from such crude lysates can then be subject to partial or complete purification before analysis.

**[0028]** Samples can be obtained from embryos, foetuses, neonatals, young infants or adults. Typically, samples are obtained from neonatals within a day, week, month or up to six months after birth. Foetal samples can be obtained in the form of, for example, amniotic fluid from the mother, or foetal blood. Amniotic fluid is preferably withdrawn from the womb of a pregnant woman using, for example, 20 or 22 gauge needles under continuous ultrasonic guidance. Methods for obtaining foetal blood are described by Daffos in The Unborn Patient-Prenatal Diagnosis and Treatment (Harrison et al. eds., W. B. Sanders, Philadelphia, PA 1991), ch. 11.

**[0029]** In some methods, multiple diagnostic tests for multiple markers are performed on the same patient. Typically, multiple tests are performed on different aliquots of the same tissue sample. However, multiple assays can also be performed on separate samples from the same tissue source, or on multiple samples from different tissue sources. For example, a test for one marker can be performed on a plasma sample, and a test for a second marker on a whole blood sample. In some methods, multiple samples are obtained from the same patient at different time points. In such methods, the multiple samples are typically from the same tissue, for example, all plasma.

### VI. Protein Assay Formats

**[0030]** Polypeptide analytes such as saposins, α-glucosidase and LAMP-1 can be assayed by a binding assay using an antibody or other reagent with specific binding affinity for the analyte. In brief, a sample containing the analyte is incubated with an antibody or other binding reagent and the complex formed is detected and quantified. Conditions for incubating an antibody or other binding reagent with a test sample vary, depending upon the format employed in the assay, the detection methods employed and the type and nature of the binding reagent used in the assay. Any one of the commonly available immunological assay formats for example radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), diffusion-based Ouchterlony, rocket-gel immunoelectrophoresis or in situ immunoassays can be used.

**[0031]** Examples of such assay formats can be found in U.S. Patent Nos. 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876, 3,791,932; 3,839,153; 3,850,752; 3,879,262; and 4,034,074, 4,016,043, 4,424.279 and 4,018,653. The results can be qualitative, by simple observations of the visible signal or can be quantitated by comparison with a control sample containing known amounts of analyte. Variations of this assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound antibody. The antibodies used above can be monoclonal or polyclonal.

**[0032]** The substrate for use in solid phase assays is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports can be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. Binding reagents can be linked to the substrate by covalent crosslinking. The binding processes generally entail cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

**[0033]** Labels for use in such assays include enzymes, fluorophores or radionuclide containing molecules (ie. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to an antibody, generally by means of glutaraldehyde or periodate. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, on hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates, which yield a fluorescent product. Alternatively, fluorescent compounds, such as fluorescein, $Eu^{3+}$ or other lanthanide metals, and rhodamine, can be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in ELISA, a fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Other reporter molecules, such as chemiluminescent or bioluminescent molecules may also be employed.

VII. Binding Reagents for Use in Protein Assay Formats

**[0034]** The above binding assays are typically performing using antibodies as the binding reagents. Antibodies can be polyclonal or monoclonal. The production of non-human monoclonal antibodies, eg., murine, rat and so forth, is well known and may be accomplished by, for example, immunising the animal with a preparation containing an analyte polypeptide (eg., a saposin, LAMP-1 or α-glucosidase) or an immunogenic fragment thereof, optionally with an adjuvant. Antibody-producing cells obtained from the immunised animals can be immortalised and screened for the production of an antibody which binds to analyte. See Harlow & Lane, Antibodies, A Laboratory Manual (C.S.H.P. NY, 1988) (incorporated by reference in its entirety for all purposes). Human and humanised antibodies can also be made but are not superior to rodent antibodies for in vitro diagnostic assays. Intact antibodies and their binding fragments, such as Fv, Fab and $(Fab')_2$ can be used in the present methods.

**[0035]** Other suitable binding reagents can be screened from random libraries of peptides or other compounds can also be screened for suitability. Combinatorial libraries can be produced for many types of compounds that can be synthesised in a step-by-step fashion. Such compounds include polypeptides, β-turn mimetics, polysaccharides, phospholipids, hormones, prostaglandins, steroids, aromatic compounds, heterocyclic compounds, benzodiazepines, oligomeric N-substituted glycines and oligocarbamates. Large combinatorial libraries of the compounds can be constructed by the encoded synthetic libraries (ESL) method described in Affymax, WO 95/12608, Affymax, WO 93/06121, Columbia University, WO 94/08051, Pharmacopeia, WO 95/35503 and Scripps, WO 95/30642 (each of which is incorporated by reference for all purposes). Peptide libraries can also be generated by phage display methods. See, eg., Devlin, WO 91/18980.

IX. mRNA Assays

**[0036]** Alternatively, or additionally to protein analyses, the invention provides methods of diagnosis based on detection and quantification of mRNA encoding saposins, LAMP-1 or α-glucosidase, or amplification products thereof. RNA transcript for analysis is isolated from a biological sample obtained from a biological tissue or fluid in which the gene of interest is expressed. Samples include sputum, blood, blood cells (eg., white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Methods of isolating total mRNA are described in Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology: Hybridisation With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier, N.Y. (1993) and Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology: Hybridisation With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier, N.Y. (1993)).

**[0037]** Frequently, it is desirable to amplify RNA prior to hybridisation. The amplification product can be RNA or DNA, single-stranded, or double-stranded. In one procedure, mRNA can be reverse transcribed with a reverse transcriptase and a primer consisting of oligo dT and a sequence encoding the phage T7 promoter to provide single stranded DNA template. The second DNA strand is polymerised using a DNA polymerase. After synthesis of double-stranded cDNA, T7 RNA polymerase is added and RNA is transcribed from the cDNA template. Successive rounds of transcription from each single cDNA template result in amplified RNA. Alternatively, cDNA can be amplified to generate double stranded amplicon, and one strand of the amplicon can be isolated, ie., using a biotinylated primer that allows capture of the undesired strand on streptavidin beads. Alternatively, asymmetric PCR can be used to generate a single-stranded target. Optionally, amplification product is labelled either in the course of amplification or subsequently. A variety of different fluorescent labels are available including fluorescein and phycoerythrin.

**[0038]** RNA or amplification products thereof are typically detected by hybridisation to a complementary probe. Either the analyte or probe can be labelled. In some assay formats, the analyte is immobilised, and in other formats the probe. Optionally, probes for multiple analytes can be immobilised on the same support and multiple analytes detected and quantified simultaneously. Methods for analysis and quantification of transcript using probe arrays are described in detail in WO 96/14839 and WO 97/01603.

**X. Methods of Diagnosis and Monitoring**

**[0039]** The analyses of levels of saposins, LAMP-1 and α-glucosidase described above have at least two principal applications. First, such analyses are useful in diagnosing patients who have not already been unequivocally characterised as having a lysosomal storage disease. Analysis of the levels of saposins, LAMP-1 and α-glucosidase provides an indication that a patient suffers from such a disease, or is susceptible to or at risk of such diseases. The relative levels of the different markers can also sometimes provide an indication that a particular lysosomal storage disorder or subset of disorders is present (see Table 1).

**TABLE 1**

| Disease | A | B | C | D | LAMP -1 | α-glu |
|---|---|---|---|---|---|---|
| Cystinosis | + | O | ++ | ++ | ND | + |
| Fabry disease | ++ | + | + | + | O | O |
| Galactosialidosis | O | O | O | O | ++ | - |
| Gaucher's disease | ++ | ++ | ++ | ++ | ++ | ++ |
| GM-1-gangliosidosis | ++ | O | + | + | ++ | O |
| I Cell | + | O | ++ | ++ | ++ | ND |
| Krabbe disease | + | O | O | O | O | + |
| α-Mannosidosis | O | O | O | + | ++ | + |
| Metachromatic Leukodystrophy | O | O | O | + | O | O |
| MPS I | + | O | ++ | ++ | ++ | O |
| MPS II | + | O | ++ | O | ++ | + |
| MPS IIIA | O | O | O | O | ++ | + |
| MPS III | O | O | O | O | ++ | O |
| MPS IIIC | O | O | O | O | ++ | + |
| MPS IIID | O | O | + | O | ++ | + |
| MPS IVA | + | O | O | O | ++ | - |
| MPS VI | + | O | + | + | ++ | O |
| Multiple Sulphatase Deficiency | O | O | ++ | ++ | ++ | ++ |
| Neuronal Ceroid Lipofuscinoses | O | O | O | O | ND | ND |
| Niemann-Pick(A/B) | ++ | ++ | ++ | ++ | O | + |
| Niemann-Pick(C) | ++ | O | ++ | ++ | O | + |
| Pompe's disease | O | + | + | ++ | O | - |
| Sandhoffs disease | ++ | + | + | O | ++ | + |
| Sialic Acid Storage disease | + | O | ++ | O | O | ++ |
| Tay-Sachs Type 1 | + | O | O | O | + | + |
| Tay-Sachs (A/B) | O | O | O | ++ | ++ | ++ |
| Wolman disease | + | O | ++ | ++ | + | ND |

**[0040]** Table 1 shows correlations between the various lysosomal storage disorders and the four saposin markers, Lamp-1 and α-glucosidase. The symbol ++ indicates a strong positive correlation (ie., at least 80% of subjects with the disorder have a level of marker of at least the 95th percentile of a control population). The symbol + indicates a lesser but still positive correlation (ie., at least 40% of subjects with the disorder have a level of marker of at least the 95th percentile in a control population. The symbol "-" indicates a negative correlation between marker levels and presence of disease. The symbol "O" is used for other measured levels. The symbol "ND" means not determined.

**[0041]** The Table shows that Gaucher's disease is the only one of the lysosomal storage disorders to show strong positive correlations with all six markers tested. A number of diseases showed a strong positive correlation for at least one saposin and for Lamp-1. These disease were, in addition to Gaucher's disease, GM-I-gangliosidosis, I cell disease, MPS I, MPS II, multiple sulphatase deficiency, Sandhoff disease and Tay-Sachs disease (A/B). Several of the diseases showed a strong positive correlation for at least one saposin and not for LAMP-1. These diseases were Niemann-Pick (A/B) and C, Pompe's disease and sialic acid storage disease. Other diseases showed a strong positive correlation for Lamp-1 and not for any saposin. These diseases included galactosialidosis, α-mannosidosis, MPS IIIA, MPS IIIB, MPS II, MPS IIIC, MPS IIID, MPS IVA, and MPS VI. Thus, by examining the levels of several or all of the markers shown in a patient and comparing with the correlations shown in Table 1 or similar Table it is possible to classify a patient as having a particular disease or subset of diseases.

**[0042]** The above diagnostic tests work by comparing a measured level of analyte in a patient with a baseline level determined in a control population of patients unaffected by a lysosomal storage disorder. A significant departure between the measured level in a patient and baseline levels in unaffected persons signals a positive outcome of the diagnostic test. A departure is considered significant if the measured value falls outside the range typically observed in unaffected individuals due to inherent variation between individuals and experimental error. For example, a departure can be considered significant if a measured level does not fall within the mean plus one standard deviation of levels in a control population. In some methods, a departure between a measured level and control levels is judged significant if the

measured level is at least the level of the, 75th, 80th or 95th percentile of a control population. In other words, the measured level in the patient occurs in only 50%, 25%, 20% or 5% of normal individuals. If the measured level of an analyte does not differ significantly from baselines levels in a control population, the outcome of the diagnostic test is considered negative.

**[0043]** For saposins and Lamp-1, a positive outcome is typically indicated by measured levels in excess of normal levels. For α-glucosidase, a positive outcome can be indicated by measured levels either in excess or below baselines levels. Whether the measured level of α-glucosidase is above or below baselines levels is indicative of the subtype of lysosomal storage disorder affecting the patient (see Table 1). The extent of departure between a measured value and a baseline value in a control population also provides an indicator of the probable accuracy of the diagnosis, and/or of the severity of the disease being suffered by the patient.

**[0044]** If a diagnostic test gives a positive outcome, the patient is, at minimum, identified as being susceptible to or at risk of a lysosomal storage disorder. The patient is then typically subject to further tests or screening. Such tests or screening can include analyses of additional analytes correlated with lysosomal storage disorders that have not already been tested. Such screening can also include performing biochemical tests for deficiency of enzymes associated with particular lysosomal storage disorders. Such assays are typically performed on urine, blood and skin fibroblasts from patients (Schriver *et al.*, supra). For example, α glucosidase can be assayed using 4-methyl-umbelliferyl-a-D-glucopyranoside as a substrate (see Van der Ploeg et al., Ped. Res. 24:90-94 (1988)). Further tests can also include monitoring for clinical symptoms of a lysosomal storage disorder, which include one or more of Dwarfism, corneal clouding, hepatosplenomegaly, valvula lesions, coronary artery lesions, skeletal deformities, joint stiffness and progressive mental retardation. Further screening can also include analyses of family history for related family members with lysosomal storage disorders, and/or genetic analyses of DNA encoding enzymes associated with such disorders from the patient for polymorphisms associated with lysosomal storage disorders. (See eg., US 5,266,459). As a result of one or more of these screening assays, the initial diagnosis based on analyte levels can be confirmed (or otherwise), and the particular lysosomal storage disorder affecting a patient can be identified.

**[0045]** Patients identified as having a particular lysosomal storage disorder are then typically administered a treatment for the disorder. Treatment is typically in the form of enzyme-replacement therapy using the enzyme that is impaired or deficient in a particular disorder. For example, Pompe's disease can be treated with α-glucosidase, as described in copending application USSN 08/700,760 filed July 29, 1996. As a further example, Gaucher's disease can be treated with glucocendorosidase. (See, eg., US 5,879,680.)

**[0046]** The second principal application of the methods lies in monitoring the condition of patients receiving treatment for a lysosomal storage disorder. A successful treatment outcome is indicated by return of analytes, such as saposins, Lamp-1, and α-glucosidase, from abnormal levels to or toward normal levels. Typically, such methods measure an initial value for the level of analyte before the patient has received treatment. Repeat measurements are then made over a period of time. If the initial level is elevated relative to the mean level in a control population, a significant reduction in level in subsequent measurements indicates a positive treatment outcome. Likewise, if the initial level of an analyte is reduced relative to the mean in a control population, a significant increase in measured levels relative to the initial level signals a positive treatment outcome. Subsequently measured levels are considered to have changed significantly relative to initial levels if a subsequent measured level differs by more than one standard deviation from the mean of repeat measurements of the initial level. If monitoring reveals a positive treatment outcome, the same treatment regime can be continued, or replaced with a treatment regime with a lower dosage. If monitoring reveals a negative treatment outcome, the previous treatment regime is typically modified, either by using a different therapeutic agent or increasing the dosage of the previous agent.

## XII. Diagnostic Kits

**[0047]** Provided are diagnostic kits containing reagents for use in the methods described above. Typically, such a kit contains at least one reagent that specifically binds to a saposin, a Lamp or α-glucosidase. Some kits contain multiple reagents specifically binding to different analytes. For example, a kit can include a reagent that specifically binds to a saposin, a reagent that specially binds to Lamp-1 and a reagent that specifically binds to α-glucosidase. Some kits contain multiple different reagents for two or more of the different saposins. For example, some kits contain a reagent that binds to saposin A without binding to other saposins, a reagent that binds to saposin C without binding to other saposins, and a reagent that binds to saposin D without binding to other saposins. The reagents are typically monoclonal or polyclonal antibodies but can be other binding agents as described above. In some kits, binding agent(s) are provided immobilised to a solid phase. Some kits provide binding reagents immobilised to the wells of a microtiter dish. Some kits further include sample(s) of the analyte(s) to be detected by the kit for use in calibration of the kit to correlate levels of signal with concentration of analyte. Some kits also contain one of the labels as described above. For example, the label can be in the form of a labelled anti-idiotypic antibody. Kits typically also include a label or instructions describing how to perform the above-described diagnostic assays, and/or how to interpret the results thereby obtained.

## Examples

### 1. Saposins as a Marker for Lysosomal Storage Disease

**[0048]** This example shows the suitability of saposins A, B, C and D as a screening marker for LSD by determining the levels of these proteins in plasma samples taken from unaffected and LSD-affected individuals.

### Materials and Methods

#### Patient Samples

**[0049]** Plasma samples used were from samples submitted to the National Referral Laboratory (Women's and Children's Hospital, Adelaide, Australia) for LSD screening and processing for routine biochemistry. Whole blood samples for fractionation studies were obtained from healthy volunteers within the laboratory.

#### Polyclonal Antibodies

**[0050]** Anti-saposins A, B, C and D polyclonal antibodies were produced and characterised as previously described [15]. Each antibody was purified on a 2-mL Hitrap™ Protein G column (Pharmacia Biotech, Uppsala, Sweden) and quantified by absorbance at 280nm (absorbance = 1.4 for 1.0g/L).

#### Europium Labelling of Polyclonal Antibodies

**[0051]** Purified anti-saposin polyclonal antibodies were labelled with $Eu^{3+}$ lanthanide, using the DELFIN® labelling kit (Wallac, North Ryde, Australia). Labelled antibodies were purified on a 1.5 x 30cm Superose 12 fast-phase liquid chromatography column (Pharmacia Biotech, Uppsala, Sweden) as previously described [13]. The amount of $Eu^{3+}$ conjugated to each antibody molecule was determined by the fluorescence of a known antibody concentration compared to a 1 nM $Eu^{3+}$ standard solution.

#### Preparation of Calibrators and Quality Control Standards

**[0052]** Liquid calibrators for the immunoquantification of saposins were prepared using purified saposin A, B, C and D proteins [20]. Purified saposin A protein was diluted in DELFIA® assay buffer to obtain final concentrations of 2.43, 1.21, 0.61, 0.3 and 0.15μg/L. For the saposin A immunoquantification assays, low and high quality control standards were prepared by diluting saposin A protein to 0.24 and 2.43 μg/L, respectively. Purified saposin B protein was diluted in the same buffer to obtain final concentrations of 6.78, 3.34, 1.67, 0.83, and 0.42μ/L For the saposin B immunoquantification assays, low and high quality control standards were prepared by diluting saposin B protein to 0.67 and 6.78μg/L, respectively. Purified saposin C protein was diluted to obtain final concentrations of 1.88, 0.94, 0.47, and 0.24 μg/L. For the saposin C immunoquantification assays, low and high quality control standards were prepared by diluting saposin C protein to 0.47 and 1.88 μg/L, respectively. Purified saposin D protein was diluted to obtain final concentrations of 0.92, 0.46, 0.23, and 0.11 μg/L For the saposin D immunoquantification assays, low and high quality control standards were prepared by diluting saposin D protein to 0.23 and 0.92 μg/L, respectively.

**[0053]** Calibrators and controls for saposins C and D were supplemented with an equal volume of control plasma sample to that being assayed, to compensate for the inhibitory effects of plasma on the saposins C and D assays. All liquid saposin calibrators and controls were stored at 4°C.

#### Immunoquantification of Saposins

**[0054]** Determination of saposins was performed using time-delayed fluorescence immunoassays. The basis of this form of assay has been outlined previously [13]. Microtiter plates (Immulon 4; Dynatech Labs., Chantilly, VA) were coated overnight at 4°C with anti-saposin polyclonal antibody (2.5mg/L diluted in 0.1M $NaHCO_3$, pH8.8, 100μL/well) and prewashed (x 1) with DELFIA® wash buffer. Samples were diluted in DELFIA® wash buffer (100μL/well), shaken for 10 min at 20°C and incubated (4h at 4°C). Plates were then washed (x 6), Eu-labelled anti-saposin polyclonal antibody added (0.25mg/L diluted in DELFIA® assay buffer, 1000μL/well) and incubated (overnight at 4°C). The plates were washed (x 6), then DELFIA® enhancement solution (200 μL) was added to each well, and the plates shaken (10 min at 2°C). The fluorescence was read on a Wallac 1234 DELFIA® Research Fluorometer. For each saposin assay, the corresponding saposin calibrators were located across the first row of the microtiter plate with the quality controls dispersed randomly. All saposin calibrators, quality controls and samples were assayed in duplicates. Saposin concen-

trations were calculated using linear regression.

**Fractionation of Whole Blood**

**[0055]** Whole blood samples, from 6 control individuals, were fractionated into plasma, white cells and red cells as previously described [21].

**Results**

**Optimisation of Saposin Immunoquantification Assays**

**[0056]** Europium labelling of anti-saposin polyclonal antibodies gave recoveries ranging from 75-100% with 4-7 $Eu^{3+}$ atoms bound to each antibody molecule. The final parameters of the immunoquantification assays were optimised by generating a series of calibration curves under different conditions tested. The parameters that were optimised included the concentrations of the coating antibodies and the Eu-labelled antibodies, and the incubation times of the antibodies and samples. Minimisation of cost, time, as well as the reproducibility of each assay was considered when selecting the final assay conditions.

**[0057]** The optimised saposins A, B and C calibration curves gave linear responses over the standard range ($R^2$ values > 0.99), however, the saposin D calibration curve had a reduced linear range ($R^2$ = 0.9575) (Figure 1). When control plasma was added to the saposins C and D calibration curves, a reduction in signal intensity was observed, 2μL of plasma gave a 20% reduction. To compensate for the inhibitory effect of plasma on the saposins C and D immunoquantification assays, control plasma at an equal volume to that being assayed was added to the calibrators. The assumption was made that LSD-affected plasma would inhibit the assays in the same manner as control plasma, therefore only control plasma was used to spike the calibrators.

**Saposin Levels in the Plasma of Control and LSD-Affected Individuals**

**[0058]** To evaluate the suitability of each of the saposins as markers for newborn screening for LSD, the levels of saposins A, B, C and D were determined in the plasma samples from 111 control individuals (median age = 7, range = 0-66) and 334 LSD-affected individuals, representing 28 different disorders (Table 1). The $95^{th}$ centiles of saposin concentrations in the control population are listed in Table 1, together with the total proportion of LSD-affected individuals having plasma saposin elevated above this level. The levels of saposins A, C and D showed a tight distribution in the plasma samples of the control population, whereas a wider range of saposin B levels were observed (Figure 2). A significant proportion of LSD-affected individuals was found to have saposin levels above the $95^{th}$ centile of the control population with some individuals having elevations of up to 10-fold the median concentration of the control population. 15 of the 28 disorders had >80% of individuals with one or more saposin levels above the $95^{th}$ centile of the control population.

Table 2. Saposins levels in plasma from control and LSD-affected individuals.

| Disorder | n | Age[a] | | Saposin Levels[b]/(% elevation)[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | A | | B | | C | | D | |
| Control | 111 | 7 | (0-66) | 12.2 | (20.2)[d] | 54.8 | (84.5) [d] | 14.6 | (25.5)[d] | 23.8 | (39.1)[d] |
| Cystinosis[e] | 9 | 6 | (1-24) | 26.7 | (56) | 57.7 | (22) | 32.6 | (89) | 49.9 | (89) |
| Fabry disease[e] | 27 | 27 | (4-47) | 33.9 | (93) | 82.5 | (48) | 29.3 | (67) | 37.9 | (48) |
| Galactosialidosis | 1 | 16 | | 15.1 | (0) | 48 | (0) | 15.2 | (0) | 25.1 | (0) |
| Gaucher disease | 52 | 19 | (0-73) | 60.3 | (96) | 124.2 | (81) | 100.2 | (94) | 127.7 | (96) |
| GM I-gangliosidosis | 12 | 3 | (0-15) | 32.5 | (83), | 72.3 | (33) | 28.1 | (67) | 35.4 | (42) |
| I-Cell disease | 16 | 7 | (0-25) | 27.3 | (69) | 47.5 | (6.3) | 53.3 | (94) | 122 | (94) |
| Krabbe disease[e] | 11 | 0.4 | (0.1-0.5) | 20.6 | (63) | 54.1 | (0) | 22.3 | (19) | 41.1 | (38) |
| α-Mannosidosis | 5 | 4 | (3-5) | 15.9 | (0) | 51 | (0) | 21.9 | (0) | 35.4 | (40) |
| Metachromatic Leukodystrophy[e] | 32 | 5 | (0-11) | 14.1 | (16) | 49.5 | (0) | 19.7 | (9.4) | 38.1 | (44) |
| MPS I | 18 | 4 | (0-29) | 23 | (67) | 56.2 | (17) | 39.2 | (83) | 47.9 | (83) |
| MPS II | 24 | 3 | (0-11) | 20.6 | (54) | 42.7 | (4.2) | 32.4 | (96) | 37.1 | (38) |
| MPS IIIA | 19 | 5 | (1-17) | 14.4 | (0) | 29 | (0) | 21.9 | (21) | 30.9 | (16) |
| MPS IIIB | 16 | 6 | (2-21) | 14.3 | (0) | 35 | (0) | 24.1 | (31) | 31.6 | (13) |
| MPS IIIC | 3 | 12 | (6-20) | 16.1 | (0) | 24.5 | (0) | 16.6 | (0) | 28.2 | (0) |
| MPS IIID | 3 | 2 | (0-3) | 17.2 | (33) | 40.7 | (0) | 27.3 | (67) | 29.8 | (0) |
| MPS IVA | 16 | 7 | (1-44) | 21.2 | (63) | 50.1 | (0) | 20.7 | (25) | 32.3 | (38) |
| MPS VI | 10 | 5 | (0-16) | 26.6 | (70) | 55.3 | (0) | 29.6 | (60) | 38 | (50) |
| Multiple Sulphatase Deficiency | 2 | 7 | (7) | 16.1 | (0) | 43.8 | (0) | 30 | (100) | 43.2 | (100) |
| Neuronal Ceroid Lipofuscinoses[e] | 1 | 4 | (4) | 8.6 | (0) | 4.7 | (0) | 20.3 | (0) | 21.5 | (0) |
| Niemann-Pick disease (AIB)[e] | 9 | 26 | (2-44) | 51.5 | (89) | 121.2 | (89) | 141.7 | (100) | 163.9 | (100) |
| Niemann-Pick disease (C)[e] | 10 | 13 | (0-40) | 24.2 | (80) | 49.3 | (30) | 50 | (100) | 82.2 | (90) |
| Pompe disease[e] | 5 | 0.3 | (0.1-0.5) | 19.5 | (40) | 44.7 | (20) | 26.8 | (60) | 43.9 | (80) |
| Sandhoff disease | 6 | 1 | (0.9-1.3) | 33.5 | (100) | 71.4 | (50) | 31.5 | (67) | 35.8 | (33) |
| Sialic Acid Storage disease | 2 | 2 | (0-3) | 20.9 | (50) | 59.6 | (0) | 36.5 | (100) | 38.2 | (0) |
| Tay-Sachs disease type 1[e] | 21 | 4 | (0-27) | 24.1 | (71) | 59.6 | (9.5) | 23.7 | (48) | 35.4 | (33) |
| Tay-Sachs disease (A/B) | 2 | 7 | (6-8) | 18.8 | (0) | 50.8 | (0) | 16.7 | (0) | 47.2 | (100) |
| Wolman disease[e] | 2 | 0.6 | (0.5-0.8) | 35 | (50) | 52.5 | (0) | 114.8 | (100) | 110.5 | (100) |

(continued)

| Disorder | n | Age[a] | Saposin Levels[b]/(% elevation)[c] | | | |
|---|---|---|---|---|---|---|
| | | | A | B | C | D |
| Total LSD Patients[d] | 334 | | (59) | (25) | (61) | (57) |

[a]Median age (range) of individuals within the group. Median saposin concentrations of individuals within the group (pill). Percentage of individuals within the group with saposin concentration above the 95[e] cenfile of the control population. [d]Saposin concentration at the 95$^{th}$ centile (μg/L). [e]Disorder that were not elevated with LAMP-1 and LAMP-2.

**[0059]** All saposin assays included low and high quality control standards in order to determine the inter-assay coefficient of variation for each assay performed with the number of replicates ranging from 17 to 24. The coefficient of variation of the saposin A assays for both the low and high quality control standards were <10%. Saposins B, C and D assays gave coefficient of variations of <10% for the high quality control standards and 15-20% for the low quality control standards. A separate experiment was carried out where 24 replicate samples of the high and low standards were assayed on a single plate in order to calculate the intra-assay coefficient of variation. Values ranging from 6-16% were obtained for the low quality control standards and <10% was obtained for the high quality control standards.

**[0060]** As LAMP-1 levels in the same plasma samples had previously been assayed [13], direct comparisons between the levels of saposins and LAMP-1 could be carried out. Using Pearson correlation, strong correlations between all saposins ($p < 0.01$) were observed, with the exception of saposin B which showed low correlation with both saposin C and D levels (Table 3). Weak correlations were observed between saposins and LAMP-1 ($p < 0.05$) and no correlations were seen between saposin concentrations and the age in the control population.

Table 3. Pearson correlation of saposins and LAMP

| | Saposin A | Saposin B | Saposin C | Saposin D | LAMP-1 |
|---|---|---|---|---|---|
| LAMP-2 | 0.316[a] | 0.101[b] | 0.394[a] | 0.341[a] | 0.863[a] |
| LAMP-1 | 0.220[a] | 0.025 | 0.271[a] | 0.273[a] | |
| Saposin D | 0.782[a] | 0.540[a] | 0.921[a] | | |
| Saposin C | 0.827[a] | 0.574[a] | | | |
| Saposin B | 0.745[a] | | | | |

[a]Correlation is significant at the 0.01 level (2-tailed). [B]Correlation is significant at the 0.05 level (2-tailed).

**Saposin Levels in Whole Blood**

**[0061]** Whole blood samples from six unaffected individuals were fractionated and the distribution of saposins was determined in plasma, white cells and red cells (Table 3). It was found that the dominant saposin in whole blood was saposin D at a concentration of 75μg/L, while a greater proportion of saposin B (77%) was found in the plasma fraction as compared to the other saposins. Saposins A, C and D distributions in plasma, white cells and red cells were relatively equal.

Table 4. Saposin Levels and Distribution in Fractionated Blood

| | Average Saposin Levels[a] (% of whole blood)[b] | | | |
|---|---|---|---|---|
| Blood Fraction | A | B | C | D |
| Whole Blood | 19.0 ± 2.9 | 25.0 ± 8.3 | 40.4 ± 7.5 | 75.3 ± 12.9 |
| Plasma | 7.2 ± 2.1 (38) | 19.2 ± 8.0 (77) | 9.4 ± 4.5 (23) | 22.6 ± 11.1 (30) |
| Red Cells | 6.8 ± 0.9 (36) | 3.2 ± 0.5(13) | 17.8 ± 2.0 (44) | 33.4 ± 8.1 (44) |
| White Cells | 4.9 ± 0.7 (25) | 2.5 ± 0.4 (10) | 13.2 ± 2.5 (33) | 19.3 ± 3.2 (26) |

[a] average saposin levels +/- standard deviation[b] percentage of saposin in whole blood present in each fraction

**Discussion**

**[0062]** In 59% of patients, saposin A was found to be elevated above the 95$^{th}$ centile of the control population, and saposins B, C and D were elevated in 25%, 61% and 57% of patients, respectively (Table 1). Of the 28 represented disorders in our study, saposin A levels in 6 groups had >80% of the individuals above the 95$^{th}$ centile of the control group, saposin B was increased in 2 groups, and saposins C and D were each elevated in 10 groups (Table 2). Together the saposins identified greater than 80% of individuals in 15 of the 28 LSD groups, 6 of these were previously not observed to be elevated with LAMP-1 [13], namely Cystinosis, Fabry disease, Niemann-Pick disease (types A/B and C), Pompe disease, and Wolman disease. The remaining LSDs not elevated with Lamp-1 (Krabbe disease, Metachromatic Leukodystrophy, and Tay-Sachs disease) had significant numbers of patients (44-71%) showing increase in saposin levels (Table 2). About 85% of LSD-affected individuals can be detected using both LAMP-1 and saposins as screening markers.

**[0063]** There was a correlation between the LSD groups that store sphingolipids or sphingolipid derivatives and those

that showed an increase in saposin levels. For example, Niemann-Pick disease (type A/B) in which sphingomyelin is stored, showed elevations in all saposins. However, of the MPS groups studied only MPS II showed saposin C elevation. Saposins also were not elevated in demyelinating disorders such as MLD and Krabbe disease. The observed elevation of saposins in the various LSD groups may therefore relate to the tissues involved in storage and to the type of substrate stored. Moreover, the degree of saposin increase may also reflect the severity of the individual.

**[0064]** The ratios of individual saposin levels in the plasma from the LSD group were also examined and compared to the control population, in order to determine whether the ratio value was a better LSD detection parameter. However, the strong correlation between the saposins (Table 3) resulted in the ratios of any two saposins giving a lower predictive value than the individual saposins.

**[0065]** Saposin B was by far the most predominant saposin in the plasma of control individuals (Table 2). A greater proportion of saposin B (77%) was found in the plasma fraction compared to saposins A, C and D (38, 23 and 30%, respectively). Saposin D is the dominant saposin in whole blood at 75 μg/L. Because saposins originate from the same precursor, their rate of synthesis should be equal. Thus, the different observed levels probably result from different half-lives, with saposin D having a longer half-life in cells, and saposin B in plasma.

**Example 2**

**[0066]** Plasma samples have been assayed for α-glucosidase protein by an immunoquantification assay using the same technology as described for the saposin study. Results are expressed as a box plot in Figure 3. Results are also presented in Table 5 showing the median level in each disorder group, and the % of patients with an α-glucosidase level elevated above the 95$^{th}$ centile of the control population. Overall 55% of patients showed an elevation of α-glucosidase. All Pompe patients showed a level of α-glucosidase below the 5$^{th}$ centile of the control population.

**[0067]** Therefore, α-glucosidase is a useful marker for the detection of a range of LSD, by detecting either an elevation or a decrease in the level of this protein in plasma serum or whole blood.

Table 5 α-glucosidase levels in plasma from control and LSD affected individuals

| | | PERCENTILES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Count | Min | Max | Mean | Sld Err | elevated[a] | 05 | 25 | 50 | 75 | 95 |
| control | 80 | 1.9 | 22.3 | 10.4 | .5 | 5 | 3.8 | 7.4 | 10.1 | 12.3 | 20.6 |
| acid lipase | 2 | 64.8 | 87.3 | 76.1 | 11.3 | 100 | | | 76.1 | . | . |
| cystinosis | 8 | 13.5 | 57.2 | 25.6 | 5.0 | 50 | | 15.7 | 21.1 | 30.4 | . |
| Fabry | 26 | 4.5 | 45.5 | 16.2 | 2.5 | 19 | 5.1 | 8.3 | 9.9 | 18.9 | 44.6 |
| Galactosialidosis | 1 | 13.8 | 13.8 | 13.8 | | 0 | 13.8 | 13.8 | 13.8 | 13.8 | 13.8 |
| Gaucher | 80 | .0 | 290.2 | 46.0 | 4.1 | 86 | 11.8 | 25.5 | 40.0 | 53.6 | 92.5 |
| GM1 | 12 | 9.6 | 29.2 | 17.3 | 1.7 | 25 | | 12.1 | 16.3 | 22.0 | . |
| Krabbe | 11 | 7.7 | 30.7 | 19.2 | 2.3 | 45 | | 13.1 | 19.5 | 24.7 | . |
| Mannosidosis | 5 | 5.8 | 24.8 | 17.6 | 3.9 | 60 | | 8.3 | 22.5 | 24.5 | |
| MLD | 31 | .7 | 55.3 | 18.5 | 1.8 | 29 | 4.0 | 12.1 | 18.0 | 21.8 | 41.7 |
| MPS I | 18 | 11.1 | 27.4 | 19.5 | 1.0 | 39 | 11.1 | 18.7 | 19.8 | 21.6 | . |
| MPS II | 24 | 13.0 | 46.1 | 26.3 | 1.8 | 71 | 13.5 | 18.0 | 26.0 | 31.9 | 44.4 |
| MPS IIIA | 19 | 8.4 | 40.1 | 27.0 | 2.2 | 79 | 8.4 | 20.9 | 26.4 | 36.9 | . |
| MPS IIIB | 16 | 10.0 | 38.3 | 21.1 | 1.8 | 44 | | 15.5 | 19.8 | 26.6 | . |
| MPS IIIC | 3 | 13.3 | 22.3 | 17.9 | 2.6 | 33 | | | 18.1 | . | . |
| MPS IIID | 3 | 6.2 | 29.4 | 20.3 | 7.2 | 66 | | | 25.4 | . | . |
| MPS IVA | 16 | 5.9 | 20.2 | 12.3 | 1.0 | 0 | | 9.0 | 13.0 | 15.6 | . |
| MPS VI | 9 | 4.7 | 65.0 | 18.9 | 5.6 | 33 | | 10.2 | 11.9 | 25.7 | . |
| MSD | 2 | 21.5 | 24.2 | 22.9 | 1.3 | 100 | | | 22.9 | . | . |
| Mucolipidosis | 16 | 7.3 | 273.2 | 134.7 | 19.4 | 88 | | 87.1 | 149.1 | 181.2 | . |
| N-P(A/B) | 9 | 12.2 | 84.0 | 38.3 | 7.9 | 67 | | 18.4 | 38.2 | 53.4 | . |
| N-P (C) | 10 | 16.3 | 84.4 | 32.1 | 6.5 | 60 | | 17.0 | 29.4 | 36.6 | . |
| Pompe | 22 | .0 | 6.3 | .9 | .3 | 0 | .0 | .2 | .6 | 1.2 | 5.7 |
| Sandhoff | 6 | 17.4 | 31.6 | 23.5 | 2.1 | 67 | | 19.7 | 22.1 | 28.5 | . |
| SAS | 16 | 18.1 | 39.7 | 26.7 | 1.8 | 88 | | 20.4 | 26.3 | 34.1 | . |
| TSD | 21 | 5.2 | 47.9 | 23.8 | 2.6 | 43 | 5.3 | 17.4 | 19.3 | 34.7 | 47.3 |
| TSD B1 | 2 | 29.0 | 35.2 | 32.1 | 3.1 | 100 | | | 32.1 | . | . |

[a]Percentage of individuals within the group with α-glucosidase concentration above the 95$^{th}$ centile of the control population.

**Example 3**

**[0068]** The experiment shown in Fig. 2 was repeated for some of the samples using a combination of monoclonal and polyclonal antibodies to saposin C. Microliter plates were coated with the monoclonal antibody 7B2 (4mg/L, 16h, 4°C), washed then incubated with plasma samples (2μL) in assay buffer (6h, 4°C). The plate was washed again, then incubated with solution-phase europium labelled antibodies as indicated at 500μg/mL (16h, 4°C). The plate was washed and developed with enhancement buffer (200μL/well) and the fluorescence read. Concentrations of saposin C were calculated based on calibration curves using recombinant saposin C.

| Plasma Sample | Detection Antibodies | | |
|---|---|---|---|
| | Poly(EU)[1] | 3A1(Eu)[2] | 3A1(Eu)/Poly(Eu) |
| Control(C2) | 4.5 | 0 | 7.8 |
| Control(C3) | 6.3 | 0 | 11.3 |
| Gaucher(614) | 46 | 0.5 | 41 |
| Gaucher(1500) | 37.5 | 4.4 | 76 |
| MLD(451) | 4.4 | 26 | 36 |
| MLD(552) | 9.4 | 39 | 46 |
| 1. Anti-saposin C polyclonal antibody 2. Anti-saposin C monoclonal antibody | | | |

**[0069]** The 7B2 antibody behaved similarly to polyclonal sera in the capture step. However, the 3A1 antibody showed decrease reactivity with control samples, decreased reactivity with Gaucher's disease samples, and increased reactivity with metachromatic leucodystrophy and neuronal ceroid lipofuscinosis samples. These results indicate that there are multiple subtypes of saposin C that are immunologically distinguishable.

**[0070]** The existence of immunologically distinguishable subtypes within the four overall classes of saposins A, B, C and D allows for more sensitive diagnostic assays using monoclonal antibodies specific to a subtype. For example, use of monoclonal antibody 3A1 offers improved sensitivity for detecting elevated levels of saposin C in metachromatic leucodystrophy and neuronal ceroid lipofuscinosis samples relative to controls. Other monoclonals to any of the saposins can be tested for increased sensitivity relative to controls using the procedure described above.

**[0071]** In some methods, a primary screen is performed using polyclonal sera to one or more of the saposins, and samples thereby identified warranting further investigation are subject to a secondary screen with monoclonal antibodies. In some methods, the monoclonal(s) used in the secondary screen are chosen because they exhibit enhanced sensitivity in detecting saposins in a particular disease state suggested by the primary screen. In some methods, a sample is tested with a plurality of monoclonals that bind the same saposin type (eg. saposin C) but specifically bind to different subtypes of this grouping. By using monoclonal antibodies specific to particular subtypes, such as 3A1, it is possible to achieve a higher level of discrimination between signals in diseased and controlled patients increasing the accuracy of the assay.

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**[0072]**

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**[0073]** Although the foregoing invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims. All publications and patent documents cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.

## Claims

**1.** A method of diagnosing or monitoring a lysosomal storage disorder in a patient, comprising:

(a) measuring a first level of at least two saposins ("first level") in a first sample obtained from the patient, wherein each saposin is different;
(b) determining the relative abundance for each saposin measured in the first level;
(c) comparing the first level to a baseline level, wherein the baseline level is a baseline of relative abundance for each saposin determined in a control population of patients unaffected by the lysosomal storage disorder; and wherein,

(i) the first level of each saposin is similar or different from the baseline level;
(ii) a difference in the relative abundance for each saposin measured in the first level is an indicator of presence or extent of the lysosomal storage disorder in the patient;
(iii) the saposins comprises saposin A, saposin B, saposin C, saposin D, prosaposin, mRNA encoding prosaposin; and

(iv) the first sample is a plasma, serum, whole blood, urine, or amniotic fluid sample.

2. The method of claim 1**,** wherein a presence of the lysosomal disorder in the patient, is indicated by the first level exceeding the baseline level.

3. The method of claim 1, further comprising:

(d) measuring a second level of at least the two saposins ("second level") in a second sample from the patient, the first and second samples being obtained at different times;
(e) determining the relative abundance for each saposin measured in the second level; and
(f) comparing the first level and the second level in the samples to monitor progression of the disease, wherein,

(i) the saposin comprises saposin A, saposin B, saposin C, saposin D prosaposin, mRNA encoding prosaposin;
(ii) the comparison of the first level and the second is an indicator of the progression of the disease in the patient; and
(iii) the second sample is a plasma, serum, whole blood, urine, or amniotic fluid sample.

4. The method of claims 1, wherein the patient is undergoing treatment for the lysosomal storage disorder.

5. The method of claim 2, wherein the first level is greater than the $95^{th}$ percentile of the baseline level in the Control population.

6. The method of claim 1, wherein the patient is not known to have a lysosomal storage disorder before the measuring step.

7. The method of.claim 1, wherein the patient is an infant less than one year old.

8. The method of claim 1, wherein the patient is a fetus and the sample is a fetal blood sample.

9. The method of claim 3, wherein a change in the first level of the saposin indicates progression or regression of the disorder in the patient that is know to have a lysosomal storage disorder.

10. The method of claim 3, wherein a change in the first level of the saposin indicates a response to treatment of the lysosomal storage disorder in the patient that being treated for the .. lysosomal storage disorder.

11. The method of claim 3, wherein the first saposin or second saposin is selected from the group consisting of saposin A, saposin B, saposin C, and saposin D.

12. The method of claim 1., wherein the saposin is selected from the group consisting of saposin A, saposin C, or saposin D.

13. The method of claim 1, wherein the measuring step comprises detecting binding between a saposin polypeptide and an antibody.

14. The method of claim 13, wherein the antibody is a monoclonal antibody.

15. The method of claim 13, wherein the antibody is immobilized to a solid phase.

16. The method of claim 1, wherein the lysosomal storage disorder is selected from the group consisting of cystinosis, Fabry's disease, Niemann-Pick disease, Pompe's disease, Wolman disease.

17. The method of claim 1, further comprising informing the patient or a parent or guardian thereof of the presence of the lysosomal storage disorder.

18. The method of claim 1, further comprising determining a treatment program based on the measurement of the first level of the first saposin.

**19.** A method of diagnosing or monitoring a lysosomal storage disorder in a patient, comprising: measuring the level of α-glucosidase in a plasma sample, or a blood sample from a patient, wherein the level is an indicator of the presence or extent of the disorder in the patient.

**20.** The method of claim 19, further comprising diagnosing the presence of a disorder selected from the group consisting of acid lipase disease, mannosidosis, MPSII, MPS IIIA, MSD, mucolipidosis, N-P (A/B), N-P (C), Sandhoff, SAS or TSD B1, if the measured level of α-glucosidase exceeds the mean level in a control population of individuals not having a lysosomal storage disease.

**21.** The method of claim 19, further comprising diagnosing the presence of disorder selected from the group consisting of galactosialidosis, MPS IVA and Pompe's disease if the measured level of α-glucosidase is below the mean level in a control population of individuals not having lysosomal storage disease.

**22.** A method of diagnosing a lysosomal storage disorder comprising measuring a level of a saposin in a first tissue sample from the patient; measuring a level of LAMP-1 or Lamp-2 in a second tissue sample from the patient; measuring a level of a α-glucosidase in a third tissue sample from the patient; wherein an increased level of saposin and/or LAMP-1 or LAMP-2, and/or an increased or decreased level of α-glucosidase in the sample relative to respective mean levels in a control population is an indicator of presence or extent of the disorder in the patient, wherein the first tissue sample, second tissue sample or third tissue sample is selected from plasma, serum, or whole blood.

**23.** A method of diagnosing Pompe's disease in a patient, comprising measuring a level of a saposin in a first tissue sample from the patient: measuring the level of α-glucosidase in a second tissue sample from the patient; wherein the presence of an increased level of the saposin and a decreased level of the α-glucosidase relative to mean levels of the saposin and α-glucosidase in a control population of individuals not having a lysosomal storage disorder indicates Pompe's disease or susceptibility thereto, wherein the first tissue sample, second tissue sample or third tissue samples is selected from plasma, serum, or whole blood.

**24.** A method of screening patients for presence of lysosomal storage disorder, comprising: measuring the level of a LAMP-1 polypeptide in a sample from the patient: measuring the level of a saposin peptide in the sample, the presence of an increased level of LAMP-1 or saposin or both relative to mean levels in a control population, indicating susceptibility to a lysosomal disorder, wherein the sample is selected from plasma, serum, or whole blood.

**25.** A diagnostic kit comprising: a first reagent that binds to a LAMP; a second reagent that binds to a saposin wherein both reagents are antibodies.

**26.** The diagnostic kit of claim 25, further comprising a third reagent that binds to an α-glucosidase.

**27.** The diagnostic kit of claim 26, wherein the first, second and third reagents are antibodies.

**28.** A method of screening a patient for presence or susceptibility to disease, comprising performing a plurality of diagnostic tests on a tissue sample from the patient for a plurality of diseases, wherein one of the diagnostic tests comprises measuring the relative level of at least two saposins and wherein the sample is selected from plasma, serum, or whole blood.

**29.** The method of claim 28, wherein a second of the diagnostic tests comprises measuring the level of LAMP-1 in the tissue sample from the patient.

**30.** The method of claim 29, wherein a third of the diagnostic tests comprises measuring the level of α-glucosidase in the tissue sample from the patient.

**31.** The method of claim 28, wherein a fourth of the diagnostic test comprises analyzing a nucleic acid encoding an enzyme associated with a lysosomal storage disorder for a polymorphic form correlated with the disorder.

**32.** A method of monitoring treatment of a lysosomal storage disease in a patient, comprising:

determining a pre-treatment relative baseline level of a at least two saposins in a sample from the patient with a lysosomal storage disorder before treatment with an agent;

determining a post-treatment relative baseline level of the saposins in a sample from the patient with the lysosomal storage disorder after treatment with the agent; and
comparing the pre-treatment relative baseline level of with the post-treatment relative baseline level of the saposins, wherein

(i) the sample is a plasma, serum, whole blood, urine, amniotic fluid sample, or a mixture of;
(ii) saposin is selected from the group consisting of saposin A, saposin B, saposin C, saposin D, prosaposin, mRNA encoding prosaposin; and,
(iii) a reduction in the post-treatment baseline level relative to the pre-treatment baseline level indicates a positive treatment outcome.

**33.** A method of monitoring treatment of acid lipase disease, mannosidosis, MPSII, MPS IIIA, MSD, mucolipidosis, N-P (A/B), N-P (C), Sandhoff, SAS or TSD B1, comprising:

determining a baseline level of an $\alpha$-glucosidase in a tissue sample from the patient with the disorder before treatment with an agent; comparing a level of the $\alpha$-glucosidase in a tissue sample from the patient with the disorder after treatment with the agent with the baseline level; wherein a decrease relative to the baseline indicates a positive treatment outcome, wherein the tissue sample is selected from plasma, serum, or whole blood.

**34.** A method of monitoring a patient with Pompe's diseases, comprising: determining a baseline level of an $\alpha$-glucosidase in a tissue sample from the patient with the disorder before treatment with the agent; comparing a level of the $\alpha$-glucosidase in a tissue sample from the patient after treatment with the agent with the baseline level; wherein an increased relative to the baseline indicates a positive treatment outcome, and wherein the tissue sample is selected from plasma, serum, or whole blood.

**Patentansprüche**

**1.** Verfahren zur Erkennung (Diagnose) oder Überwachung einer lysosomalen Speicherkrankheit bei einem Patienten, umfassend:

a) Messen eines ersten Wertes (Level) von mindestens zwei Saposinen ("erster Wert") in einer ersten vom Patienten erhaltenen Probe, wobei jedes Saposin unterschiedlich ist;
b) Ermitteln der relativen Häufigkeit für jedes beim ersten Wert gemessene Saposin;
c) Vergleichen des ersten Wertes mit einem Basiswert; wobei der Basiswert die Basis der relativen Häufigkeit für jedes aus einer Kontrollgruppe von Patienten, welche nicht von einer lysosomalen Speicherkrankheit betroffen sind, ermittelte Saposin ist; und
wobei

i) der erste Wert jedes Saposins gleich oder verschieden von dem Basiswert ist;
ii) eine Differenz der relativen Häufigkeit für jedes beim ersten Wert gemessene Saposin ein Indikator für das Vorhandensein oder das Ausmaß der lysosomalen Speicherkrankheit bei dem Patienten ist;
iii) die Saposine Saposin A, Saposin B, Saposin C, Saposin D, Prosaposin und Prosaposin kodierende mRNA umfassen;
iv) die Probe ein Plasma, ein Serum, Vollblut, Urin oder Fruchtwasser ist.

**2.** Verfahren nach Anspruch 1, wobei ein Vorliegen der lysosomalen Speicherkrankheit durch einen den Basiswert überschreitenden ersten Wert bezeichnet wird.

**3.** Verfahren nach Anspruch 1, weiterhin umfassend:

d) Messen eines zweiten Wertes (Level) von mindestens zwei Saposinen ("zweiter Wert") in einer zweiten vom Patienten erhaltenen Probe, wobei die erste und die zweite Probe zu unterschiedlichen Zeitpunkten entnommen wurden;
e) Ermitteln der relativen Häufigkeit für jedes beim zweiten Wert gemessene Saposin; und
f) Vergleichen des ersten Wertes mit dem zweiten Wert der Proben, um den Verlauf der Krankheit zu überwachen, wobei

i) die Saposine Saposin A, Saposin B, Saposin C, Saposin D, Prosaposin und Prosaposin kodierende mRNA umfassen;
ii) der Vergleich zwischen dem ersten Wert und dem zweiten Wert ein Indikator für den Verlauf der Krankheit bei dem Patient ist; und
iii) die zweite Probe ein Plasma, ein Serum, Vollblut, Urin oder Fruchtwasser ist.

**4.** Verfahren nach Anspruch 1, wobei der Patient in Behandlung für eine lysosomale Speicherkrankheit ist.

**5.** Verfahren nach Anspruch 2, wobei der erste Wert größer als die 95. Perzentile des Ausgangswertes der Kontrollgruppe ist.

**6.** Verfahren nach Anspruch 1, wobei es vor der Durchführung der Meßschritte nicht bekannt ist, ob der Patient an einer lysosomalen Speicherkrankheit erkrankt ist.

**7.** Verfahren nach Anspruch 1, wobei der Patient ein Säugling ist, der weniger als ein Jahr alt ist.

**8.** Verfahren nach Anspruch 1, wobei der Patient ein Fötus ist und die Probe eine fötale Blutprobe ist.

**9.** Verfahren nach Anspruch 3, wobei eine Änderung des ersten Wertes der Saposinen ein Fortschreiten oder einen Rückgang der Krankheit bei dem Patienten anzeigt, der an einer lysosomalen Speicherkrankheit erkrankt ist.

**10.** Verfahren nach Anspruch 3, wobei eine Änderung des ersten Wertes der Saposinen eine Reaktion auf die Behandlung der lysosomalen Speicherkrankheit bei einem Patienten anzeigt, der an einer lysosomalen Speicherkrankheit erkrankt ist.

**11.** Verfahren nach Anspruch 3, wobei das erste oder das zweite Saposin aus der Gruppe, bestehend aus Saposin A, Saposin B, Saposin C und Saposin D, ausgewählt wird.

**12.** Verfahren nach Anspruch 1, wobei das Saposin aus einer Gruppe, bestehend aus Saposin A, Saposin C oder Saposin D, ausgewählt wird.

**13.** Verfahren nach Anspruch 1, wobei der Meßschritt das Erfassen einer Bindung zwischen einem Saposin-Polypeptid und einem Antikörper beinhaltet.

**14.** Verfahren nach Anspruch 13, wobei der Antikörper ein monoklonaler Antikörper ist.

**15.** Verfahren nach Anspruch 13, wobei der Antikörper an einer festen Phase immobilisiert ist.

**16.** Verfahren nach Anspruch 1, wobei die lysosomale Speicherkrankheit aus der Gruppe, bestehend aus Cystinose, Fabry-Krankheit, Niemann-Pick Krankheit, Morbus Pompe, Morbus Wolman ausgewählt wird.

**17.** Verfahren nach Anspruch 1, weiter umfassend:

Informieren des Patienten, eines Elternteils oder eines Erziehungsberechtigten über das Vorliegen einer lysosomalen Speicherkrankheit.

**18.** Verfahren nach Anspruch 1, weiter umfassend:

Bestimmen eines Behandlungsplans auf Basis der Messung des ersten Wertes des ersten Saposins.

**19.** Verfahren zur Erkennung (Diagnose) oder Überwachung einer lysosomalen Speicherkrankheit bei einem Patienten umfassend:

Messen des Wertes (Level) der ⟨-Glukosidase in einer Plasma- oder Blutprobe des Patienten, wobei der Wert ein Indikator für das Vorliegen oder das Ausmaß der Krankheit bei dem Patienten ist.

**20.** Verfahren nach Anspruch 19, weiter umfassend:

Erkennen (Diagnose) des Vorliegens der Krankheit, ausgewählt aus der Gruppe, umfassend Defizienz der sauren Lipase, Mannosidose, MPS II, MPS IIIA, MSD, Mucolipidose, Niemann-Pick (A/B), Niemann-Pick (C), Sandhoff-Krankheit, SAS oder TSD BI, wenn der gemessene Wert der ⟨-Glukosidase den Durchschnittswert einer Kontrollgruppe von Patienten, die nicht von einer lysosomalen Speicherkrankheit betroffen sind, überschreitet.

**21.** Verfahren nach Anspruch 19, weiter umfassend:

Erkennen (Diagnose) des Vorliegens einer Krankheit ausgewählt aus der Gruppe, umfassend Galaktosialidose, MPS IVA und Morbus Pompe, wenn der gemessene Wert der ⟨-Glukosidase den Durchschnittswert einer Kontrollgruppe von Patienten, die nicht von einer lysosomalen Speicherkrankheit betroffen sind, unterschreitet.

**22.** Verfahren zur Erkennung (Diagnose) einer lysosomalen Speicherkrankheit, umfassend:

Messen des Wertes (Level) des Saposins in einer ersten Gewebeprobe des Patienten;
Messen des Wertes (Level) einer LAMP-1 oder LAMP-2 in einer zweiten Gewebeprobe des Patienten;
Messen des Wertes (Level) der <-Glukosidase in einer dritten Gewebeprobe des Patienten;
wobei ein erhöhter Wert des Saposins und/oder LAMP-1 oder LAMP-2 und/oder ein erhöhter oder verringerter Wert der ⟨-Glukosidase in der Probe im Vergleich zu den Durchschnittswerten einer Kontrollgruppe ein Indikator für das Vorliegen oder das Ausmaß der Krankheit bei einem Patienten ist,
wobei die erste Gewebeprobe, die zweite Gewebeprobe oder die dritte Gewebeprobe aus Plasma, Serum oder Vollblut ausgewählt wird.

**23.** Verfahren zur Erkennung (Diagnose) von Morbus Pompe bei einem Patienten, umfassend:

Messen des Wertes (Level) des Saposins in einer ersten Gewebeprobe des Patienten;
Messen des Wertes (Level) der $\alpha$-Glukosidase in einer zweiten Gewebeprobe des Patienten,
wobei das Vorliegen eines erhöhten Wertes des Saposins und eines verringerten Wertes der <-Glukosidase im Vergleich zu den Durchschnittswerten von Saposin und <-Glukosidase einer Kontrollgruppe von Patienten, die nicht von einer lysosomalen Krankheit betroffen sind, auf eine Morbus Pompe oder eine Anfälligkeit dafür hindeutet,
wobei die erste Gewebeprobe, die zweite Gewebeprobe oder die dritte Gewebeprobe aus Plasma, Serum oder Vollblut ausgewählt wird.

**24.** Verfahren zur Untersuchung von Patienten auf das Vorliegen einer lysosomalen Speicherkrankheit, umfassend:

Messen des Wertes (Level) des LAMP-1-Polypeptid in einer Probe des Patienten;
Messen des Wertes (Level) des Saposin-Polypeptid in der Probe,
wobei das Vorliegen eines erhöhten Wertes des LAMP-1 oder des Saposins im Vergleich zu den Durchschnittswerten einer Kontrollgruppe auf eine Anfälligkeit für lysosomale Krankheiten hindeutet,
wobei die Probe aus Plasma, Serum oder Vollblut ausgewählt wird.

**25.** Diagnoseausrüstung (Diagnose-Kit) umfassend ein erstes Reagenz, das an LAMP bindet, und ein zweites Reagenz, das an Saposin bindet, wobei beide Reagenzien Antikörper sind.

**26.** Diagnoseausrüstung nach Anspruch 25, weiter umfassend ein drittes Reagenz, das an eine ⟨-Glukosidase bindet.

**27.** Diagnoseausrüstung nach Anspruch 26, wobei das erste, das zweite und das dritte Reagenz Antikörper sind.

**28.** Verfahren zur Untersuchung eines Patienten auf das Vorliegen einer Krankheit oder auf die Anfälligkeit für eine Krankheit, umfassend die Durchführung einer Vielzahl von diagnostischen Untersuchungen an Gewebeproben des Patienten auf eine Vielzahl von Krankheiten, wobei eine Untersuchung die Messung der relativen Werte (Level) von mindestens zwei Saposinen umfaßt und die Probe aus Plasma, Serum oder Vollblut ausgewählt wird.

**29.** Verfahren nach Anspruch 28, wobei eine zweite diagnostische Untersuchung die Messung des Wertes (Level) von LAMP-1 in einer Gewebeprobe des Patienten umfaßt.

**30.** Verfahren nach Anspruch 29, wobei eine dritte diagnostische Untersuchung die Messung des Wertes (Level) der

<-Glukosidase in einer Gewebeprobe des Patienten umfaßt.

**31.** Verfahren nach Anspruch 28, wobei eine vierte diagnostische Untersuchung die Analyse einer Nukleinsäure, die für ein mit einer lysosomalen Speicherkrankheit zusammenhängendes Enzym kodiert, auf eine polymorphe, mit der Krankheit zusammenhängende Form umfaßt.

**32.** Verfahren zur Überwachung der Behandlung der lysosomalen Speicherkrankheit bei Patienten, umfassend:

Bestimmen eines relativen Basiswertes von mindestens zwei Saposinen in einer Probe eines Patienten mit der lysosomalen Speicherkrankheit vor einer Behandlung mit einem Erreger;
Bestimmen eines relativen Basiswertes von mindestens zwei Saposinen in einer Probe eines Patienten mit der lysosomalen Speicherkrankheit nach einer Behandlung mit einem Erreger; und
Vergleichen des relativen Basiswertes vor der Behandlung mit dem relativen Basiswert der Saposinen nach der Behandlung,
wobei

i) die Probe aus Plasma, Serum, Vollblut, Urin, Fruchtwasser oder einer Mischung daraus besteht;
ii) das Saposin aus der Gruppe, bestehend aus Saposin A, Saposin B, Saposin C, Saposin D, Prosaposin und Prosaposin kodierender mRNA ausgewählt wird; und
iii) eine Verringerung des relativen Basiswerts nach der Behandlung im Vergleich zum relativen Basiswert vor der Behandlung auf ein positives Behandlungsergebnis schliessen lässt.

**33.** Verfahren zur Überwachung der Behandlung von Defizienz der sauren Lipase, Mannosidose, MPSII, MPS IIIA, MDS, Mucolipidose, Niemann-Pick (A/B), Niemann-Pick (C), Sandhoff-Krankheit, SAS oder TSD B1, umfassend:

Bestimmen des Basiswertes der <-Glukosidase in einer Gewebeprobe des Patienten mit der Krankheit, bevor dieser mit einem Errerger behandelt wird;
Vergleichen des Wertes der <-Glukosidase in der Gewebeprobe des Patienten mit der Krankheit, nachdem dieser mit einem Erreger behandelt worden ist, mit dem Basiswert, wobei eine Verringerung im Vergleich zum Basiswert auf ein positives Behandlungsergebnis deutet, und
wobei die Gewebeprobe aus Plasma, Serum oder Vollblut ausgewählt wird.

**34.** Verfahren zur Überwachung eines Patienten mit Morbus Pompe, umfassend:

Bestimmen des Basiswertes der <-Glukosidase in einer Gewebeprobe eines Patienten mit der Krankheit, bevor dieser mit einem Erreger behandelt wird;
Vergleichen des Wertes der <-Glukosidase in einer Gewebeprobe eines Patienten, nachdem dieser mit einem Erreger behandelt worden ist, wobei eine Erhöhung im Vergleich zum Basiswert auf ein positives Behandlungsergebnis deutet, und
wobei die Gewebeprobe aus Plasma, Serum oder Vollblut ausgewählt wird.

**Revendications**

**1.** Procédé de diagnostic ou de surveillance d'un trouble de surcharge lysosomale chez un patient, qui comprend :

(a) la mesure d'un premier niveau d'au moins deux saposines (« premier niveau ») dans un premier échantillon obtenu du patient, dans lequel chaque saposine est différente ;
(b) la détermination de l'abondance relative de chaque saposine mesurée dans le premier niveau ;
(c) la comparaison du premier niveau à un niveau de base, le niveau de base étant une valeur de base de l'abondance relative de chaque saposine déterminée dans une population de contrôle de patients non affectés par le trouble de surcharge lysosomale ; et
dans lequel,

(i) le premier niveau de chaque saposine est similaire ou différent du niveau de base ;
(ii) une différence dans l'abondance relative de chaque saposine mesurée dans le premier niveau est un indicateur de la présence ou du degré de trouble de surcharge lysosomale chez le patient ;
(iii) la saposine comprend la saposine A, la saposine B, la saposine C, la saposine D, la prosaposine,

l'ARNm codant pour la prosaposine ; et
(iv) le premier échantillon est un échantillon de plasma, de sérum, de sang total, d'urine, ou de liquide amniotique.

**2.** Procédé selon la revendication 1, dans lequel une présence du trouble lysosomal chez le patient est indiquée par le fait que le premier niveau dépasse le niveau de base.

**3.** Procédé selon la revendication 1, comprenant en outre :

(d) la mesure d'un deuxième niveau d'au moins les deux saposines (« deuxième niveau ») dans un deuxième échantillon du patient, les premier et deuxième échantillons étant obtenus à différents moments ;
(e) la détermination de l'abondance relative de chaque saposine mesurée dans le deuxième niveau ; et (f) la comparaison du premier niveau et du deuxième niveau dans les échantillons pour surveiller la progression de la maladie,
dans lequel,

(i) la saposine comprend la saposine A, la saposine B, la saposine C, la saposine D, la prosaposine, l'ARNm codant pour la prosaposine ;
(ii) la comparaison du premier niveau et du deuxième niveau est un indicateur de la progression de la maladie chez le patient ; et
(iii) le deuxième échantillon est un échantillon de plasma, de sérum, de sang total, d'urine ou de liquide amniotique.

**4.** Procédé selon la revendication 1, dans lequel le patient subit un traitement pour le trouble de surcharge lysosomale.

**5.** Procédé selon la revendication 2, dans lequel le premier niveau est supérieur au 95$^{ème}$ percentile du niveau de base dans la population de contrôle.

**6.** Procédé selon la revendication 1, dans lequel on ne sait pas si le patient souffre d'un trouble de surcharge lysosomale avant l'étape de mesure.

**7.** Procédé selon la revendication 1, dans lequel le patient est un enfant de moins d'un an.

**8.** Procédé selon la revendication 1, dans lequel le patient est un foetus et l'échantillon est un échantillon de sang fatal.

**9.** Procédé selon la revendication 3, dans lequel une modification du premier niveau de saposine indique la progression ou la régression du trouble chez le patient dont on sait qu'il souffre d'un trouble de surcharge lysosomale.

**10.** Procédé selon la revendication 3, dans lequel une modification du premier niveau de la saposine indique une réponse au traitement du trouble de surcharge lysosomale chez le patient traité pour le trouble de surcharge lysosomale.

**11.** Procédé selon la revendication 3, dans lequel la première saposine ou la deuxième saposine est choisie dans le groupe constitué par la saposine A, la saposine B, la saposine C, et la saposine D.

**12.** Procédé selon la revendication 1, dans lequel la saposine est choisie dans le groupe constitué par la saposine A, la saposine C, ou la saposine D.

**13.** Procédé selon la revendication 1, dans lequel l'étape de mesure comprend la détection de la liaison entre un polypeptide de saposine et un anticorps.

**14.** Procédé selon la revendication 13, dans lequel l'anticorps est un anticorps monoclonal.

**15.** Procédé selon la revendication 13, dans lequel l'anticorps est immobilisé sur une phase solide.

**16.** Procédé selon la revendication 1, dans lequel le trouble de surcharge lysosomale est choisi dans le groupe constitué par la cystinose, la maladie de Fabry, la maladie de Niemann-Pick, la maladie de Pompe, la maladie de Wolman.

**17.** Procédé selon la revendication 1, comprenant en outre le fait d'informer le patient ou un parent ou un tuteur de

celui-ci de la présence du trouble de surcharge lysosomale.

**18.** Procédé selon la revendication 1, comprenant en outre la détermination d'un programme de traitement basé sur la mesure du premier niveau de la première saposine.

**19.** Procédé de diagnostic ou de surveillance d'un trouble de surcharge lysosomale chez un patient, qui comprend :

la mesure du niveau d'α-glucosidase dans un échantillon de plasma, ou un échantillon de sang d'un patient, dans lequel le niveau est un indicateur de la présence ou du degré du trouble chez le patient.

**20.** Procédé selon la revendication 19, comprenant en outre le diagnostic de la présence d'un trouble choisi dans le groupe constitué par une maladie liée à la lipase acide, la mannosidose, la MPSII, la MPS IIIA, la MSD, la mucolipidose, la N-P (A/B), la N-P (C), la maladie de Sandhoff, une SAS ou la TSD B1, si le niveau mesuré d'α-glucosidase dépasse le niveau moyen dans une population de contrôle d'individus ne souffrant pas de maladie de surcharge lysosomale.

**21.** Procédé selon la revendication 19, comprenant en outre le diagnostic de la présence d'un trouble choisi dans le groupe constitué par la galactosialidose, la MPS IVA et la maladie de Pompe si le niveau mesuré d'α-glucosidase est inférieur au niveau moyen dans une population de contrôle d'individus ne souffrant pas de trouble de surcharge lysosomale.

**22.** Procédé de diagnostic d'un trouble de surcharge lysosomale qui comprend la mesure d'un niveau d'une saposine dans un premier échantillon de tissu du patient ; la mesure d'un niveau de LAMP-1 ou de LAMP-2 dans un deuxième échantillon de tissu du patient ; la mesure d'un niveau d'α-glucosidase dans un troisième échantillon de tissu du patient ; dans lequel un niveau plus élevé de saposine et/ou de LAMP-1 ou de LAMP-2, et/ou un niveau plus élevé ou moins élevé d'α-glucosidase dans l'échantillon par rapport aux niveaux moyens respectifs dans une population de contrôle est un indicateur de la présence ou du degré de trouble chez le patient, dans lequel le premier échantillon de tissu, le deuxième échantillon de tissu ou le troisième échantillon de tissu est choisi parmi un échantillon de plasma, de sérum, ou de sang total.

**23.** Procédé de diagnostic de la maladie de Pompe chez un patient, qui comprend la mesure d'un niveau de saposine dans un premier échantillon de tissu du patient ; la mesure du niveau d'α-glucosidase dans un deuxième échantillon de tissu du patient ; dans lequel la présence d'un niveau plus élevé de la saposine et d'un niveau moins élevé de l'α-glucosidase par rapport à des niveaux moyens de al saposine et de l'α-glucosidase dans une population de contrôle d'individus ne souffrant pas de trouble de surcharge lysosomale indique la maladie de Pompe ou une prédisposition à celle-ci, dans lequel le premier échantillon de tissu, le deuxième échantillon de tissu ou le troisième échantillon de tissu est choisi parmi un échantillon de plasma, de sérum, ou de sang total.

**24.** Procédé de criblage de patients à la recherche de la présence d'un trouble de surcharge lysosomale, qui comprend : la mesure du niveau d'un polypeptide LAMP-1 dans un échantillon du patient ; la mesure du niveau d'un peptide de saposine dans l'échantillon, la présence d'un niveau plus élevé de LAMP-1 ou de saposine ou des deux relativement à des niveaux moyens dans une population de contrôle indiquant la prédisposition à un trouble lysosomal, dans lequel l'échantillon est choisi parmi un échantillon de plasma, de sérum, ou de sang total.

**25.** Kit de diagnostic qui comprend : un premier réactif qui se lie à un LAMP ; un deuxième réactif qui se lie à une saposine ; dans lequel les deux réactifs sont des anticorps.

**26.** Kit de diagnostic selon la revendication 25, comprenant en outre un troisième réactif qui se lie à une α-glucosidase.

**27.** Kit de diagnostic selon la revendication 26, dans lequel les premier, deuxième et troisième réactifs sont des anticorps.

**28.** Procédé de criblage d'un patient à la recherche de la présence ou de la susceptibilité à une maladie, qui comprend la réalisation d'une pluralité de tests diagnostiques sur un échantillon de tissu du patient pour une pluralité de maladies, dans lequel un des tests diagnostiques comprend la mesure du niveau relatif d'au moins deux saposines, dans lequel l'échantillon est choisi parmi un échantillon de plasma, de sérum, ou de sang total.

**29.** Procédé selon la revendication 28, dans lequel un deuxième test diagnostique comprend la mesure du niveau de LAMP-1 dans l'échantillon de tissu du patient.

**30.** Procédé selon la revendication 29, dans lequel un troisième test diagnostique comprend la mesure du niveau d'α-glucosidase dans l'échantillon de tissu du patient.

**31.** Procédé selon la revendication 28, dans lequel un quatrième test diagnostique comprend l'analyse d'un acide nucléique codant pour une enzyme associée à une maladie de surcharge lysosomale pour une forme polymorphe corrélée au trouble.

**32.** Procédé de surveillance du traitement d'un trouble de surcharge lysosomale chez un patient, qui comprend :

la détermination d'un niveau de base relatif prétraitement d'au moins deux saposines dans un échantillon du patient souffrant d'un trouble de surcharge lysosomale avant le traitement avec un agent ;
la détermination d'un niveau de base relatif post-traitement des saposines dans un échantillon du patient souffrant d'un trouble de surcharge lysosomale après le traitement avec l'agent ; et
la comparaison du niveau de base relatif prétraitement au niveau de base relative post-traitement des saposines, dans lequel :

(i) l'échantillon est un échantillon de plasma, de sérum, de sang total, d'urine, de liquide amniotique, ou un mélange de ceux-ci ;
(ii) la saposine est choisie dans le groupe constitué par la saposine A, la saposine B, la saposine C, la saposine D, la prosaposine, l'ARNm codant pour la prosaposine ; et (iii) une diminution du niveau de base post-traitement par rapport au niveau de base prétraitement indique un résultat de traitement positif.

**33.** Procédé de surveillance d'une maladie liée à la lipase acide, de la mannosidose, de la MPSII, de la MPS IIIA, de la MSD, de la mucolipidose, de la N-P (A/B), de la N-P (C), de la maladie de Sandhoff, d'une SAS ou de la TSD B1, qui comprend : la détermination d'un niveau de base d'une α-glucosidase dans un échantillon de tissu d'un patient souffrant du trouble avant le traitement avec un agent ; la comparaison d'un niveau de base de l'α-glucosidase dans un échantillon de tissu du patient souffrant du trouble après le traitement avec un agent au niveau de base ; dans lequel une diminution par rapport à la valeur de base indique un résultat de traitement positif, dans lequel l'échantillon de tissu est choisi parmi un échantillon de plasma, de sérum, ou de sang total.

**34.** Procédé de surveillance d'un patient souffrant de la maladie de Pompe, qui comprend : la détermination d'un niveau de base d'une α-glucosidase dans un échantillon de tissu du patient souffrant du trouble avant le traitement avec l'agent ; la comparaison d'un niveau de base de l'a-glucosidase dans un échantillon de tissu du patient après le traitement avec l'agent au niveau de base ; dans lequel une augmentation par rapport à la valeur de base indique un résultat de traitement positif, et dans lequel l'échantillon de tissu est choisi parmi un échantillon de plasma, de sérum, ou de sang total.

## Figure 1

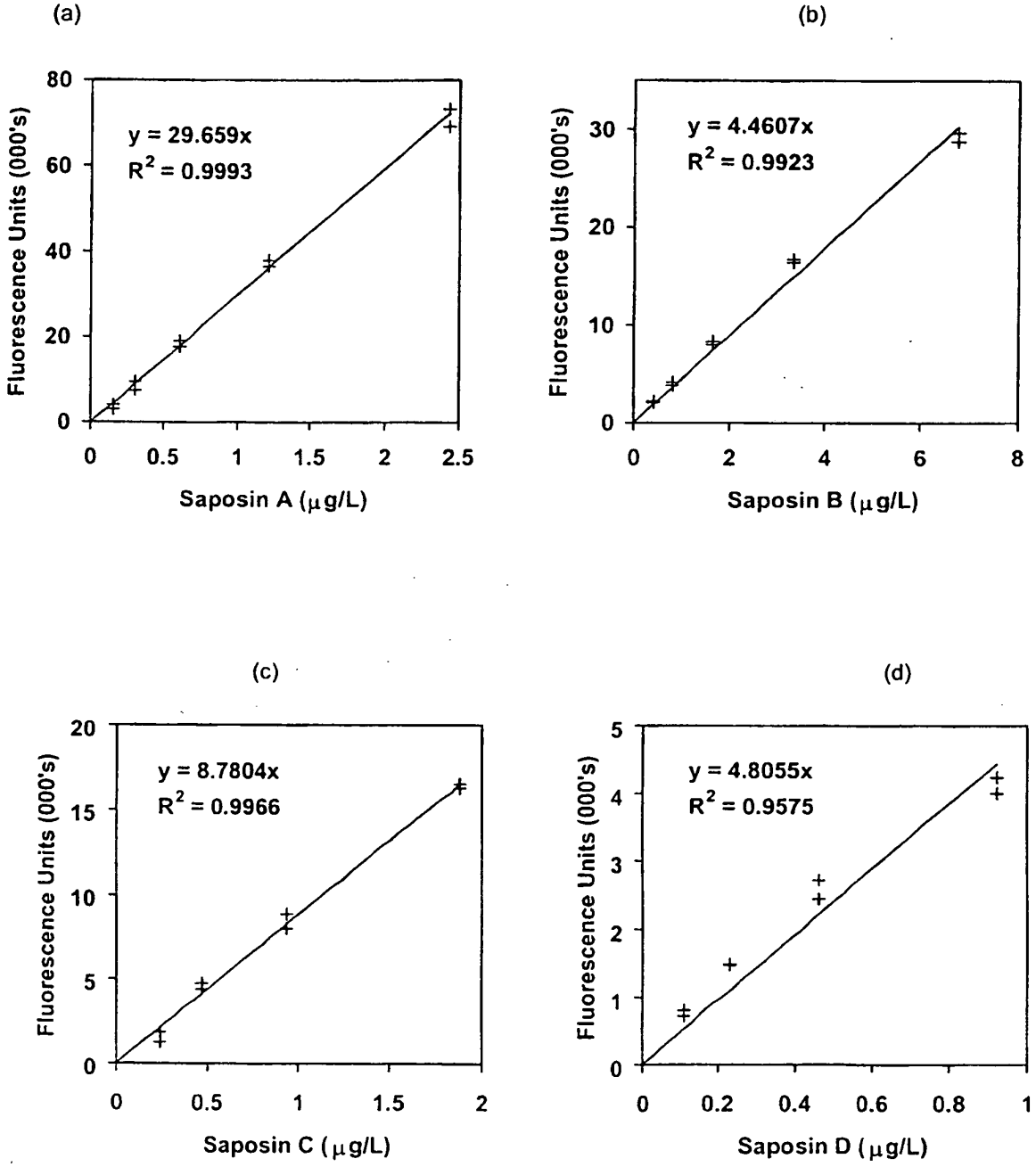

(a)
Fluorescence Units (000's)
y = 29.659x
R2 = 0.9993
Saposin A (µg/L)
(b)
Fluorescence Units (000's)
y = 4.4607x
R2 = 0.9923
Saposin B (µg/L)
(c)
Fluorescence Units (000's)
y = 8.7804x
R2 = 0.9966
Saposin C (µg/L)
(d)
Fluorescence Units (000's)
y = 4.8055x
R2 = 0.9575
Saposin D (µg/L)

Figure 2, Sheet 1

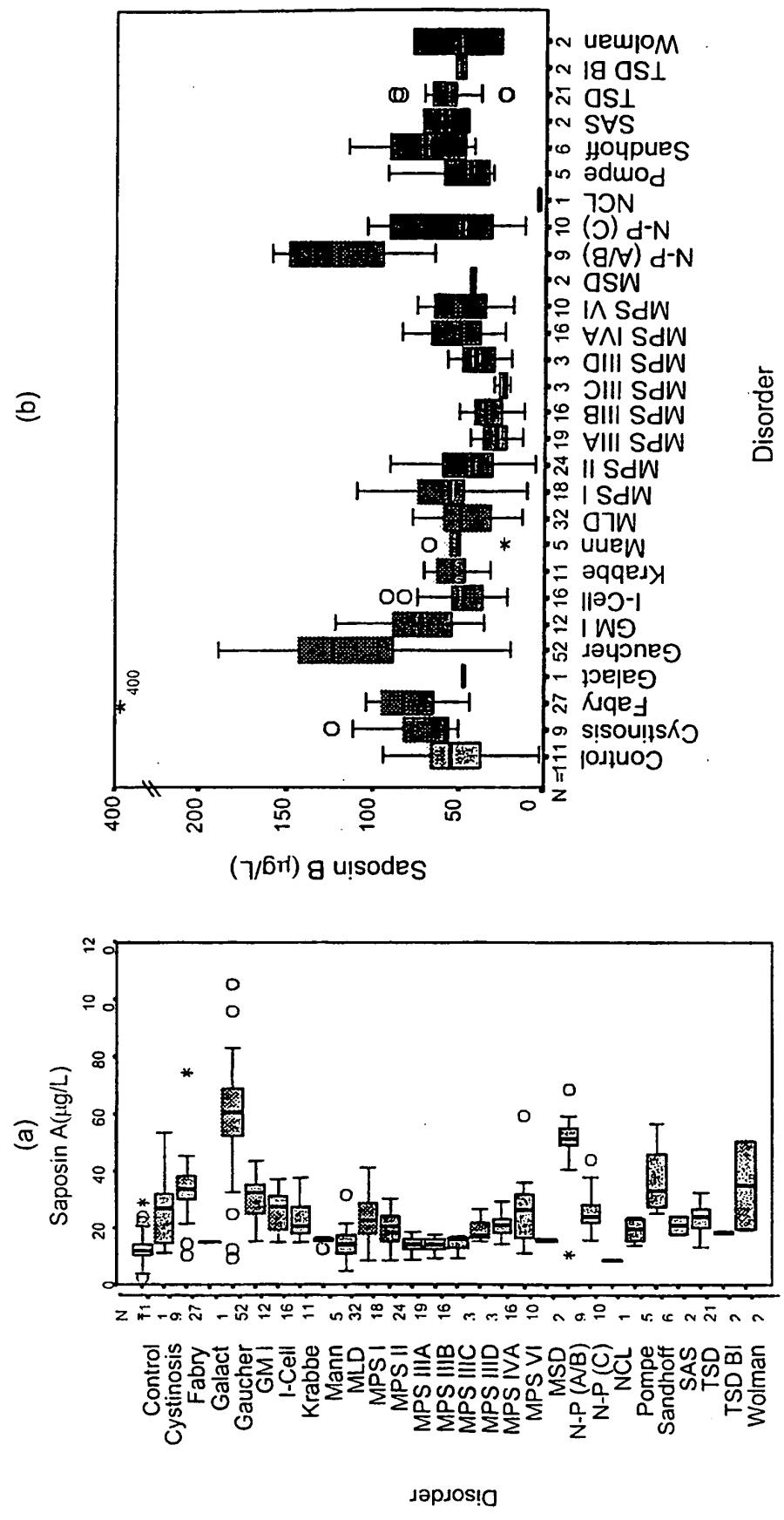
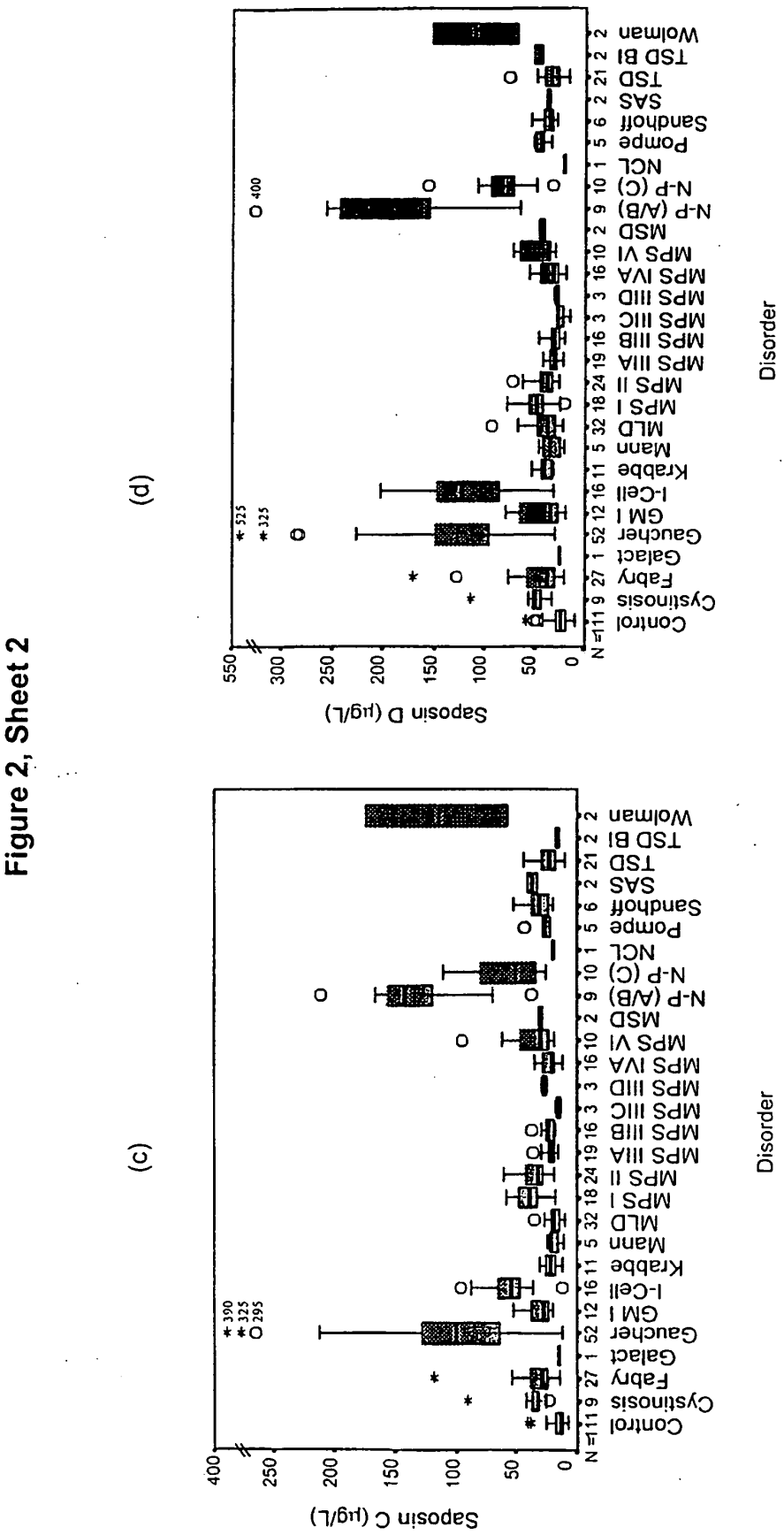


Figure 2, Sheet 2



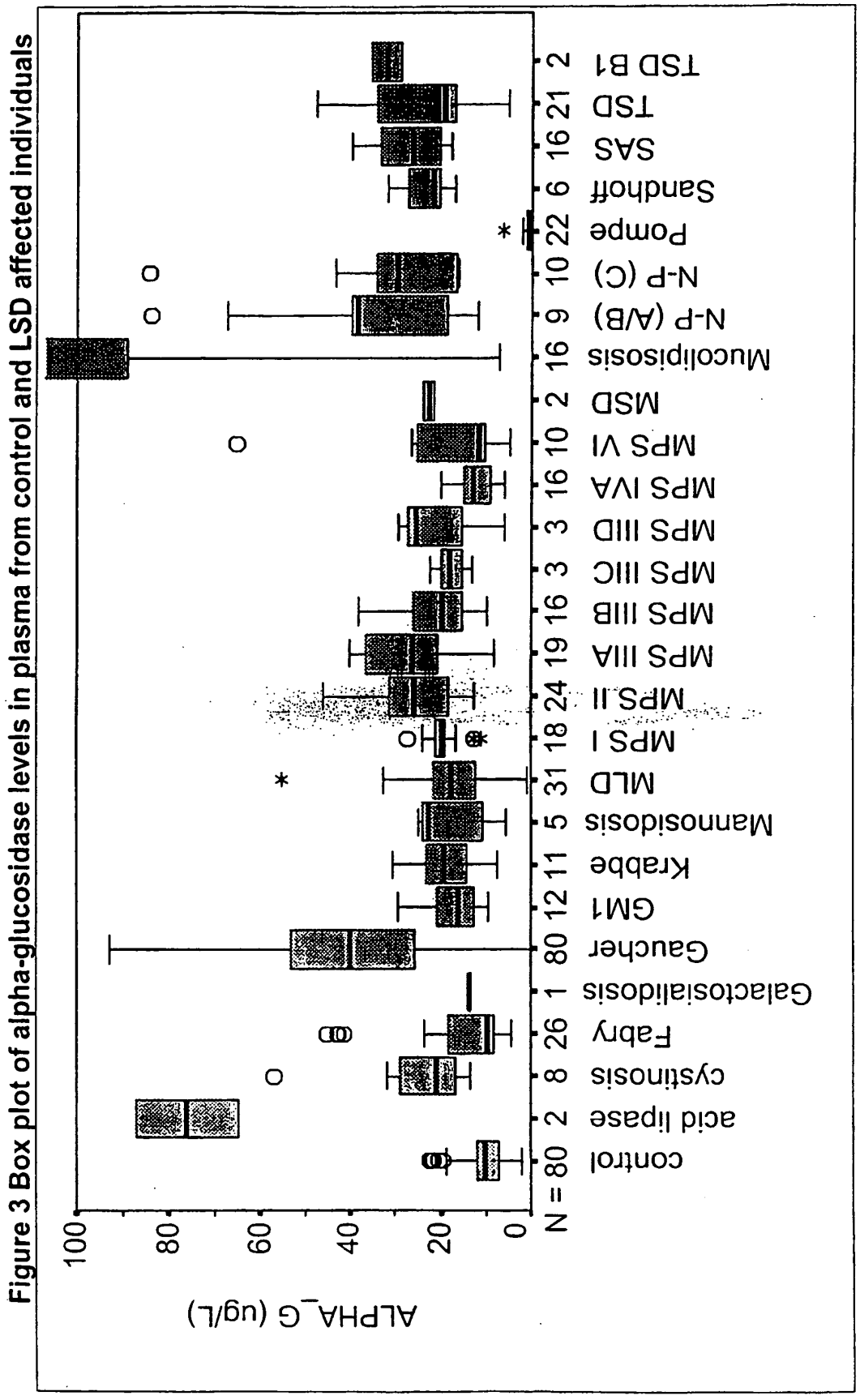



Figure 3 Box plot of alpha-glucosidase levels in plasma from control and LSD affected individuals

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| 专利名称(译) | 使用鞘脂激活蛋白和其他标志物诊断溶酶体贮积症 | | |
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摘要(译)

本发明提供了基于检测患者样品中鞘脂激活蛋白，LAMP和/或α-葡糖苷酶水平来诊断或监测溶酶体贮积症的方法。鞘脂激活蛋白和/或LAMP水平升高是一种疾病的指征。升高的α葡糖苷酶水平指示某些类型的溶酶体贮积症，并且降低的α葡糖苷酶水平指示其他类型的溶酶体贮积症。在一些方法中，不同鞘脂激活蛋白，LAMP和α葡糖苷酶的升高谱允许区分不同类型的溶酶体贮积症。