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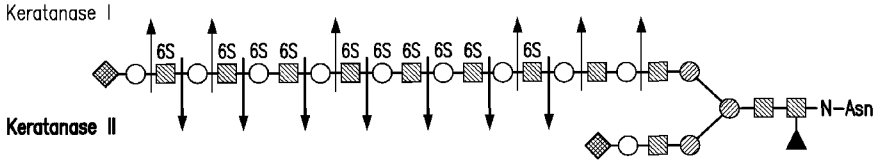


FIG. 1

(57) Abstract: Provided herein are processes for detecting oligosaccharides in a biological sample. In specific instances, the biological sample is provided from an individual suffering from a disorder associated with abnormal glycosaminoglycan accumulation.

DETECTION OF OLIGOSACCHARIDES

BACKGROUND OF THE INVENTION

[0001] Glycosaminoglycans comprise a reducing end and a non-reducing end. Disorders associated with abnormal glycosaminoglycan degradation, biosynthesis, and/or accumulation can result in an accumulation of abnormal glycosaminoglycans and fragments thereof.

SUMMARY OF THE INVENTION

[0002] In one aspect, described herein are populations of glycosaminoglycans (e.g., normal or abnormal keratan sulfates, chondroitin sulfates, and dermatan sulfates) that are transformed into populations of oligosaccharides using glycosaminoglycan digesting enzymes, e.g., lyases. Further described herein are the use of analytical instruments to characterize the population of oligosaccharides in order to provide relevant information about the population of oligosaccharides, the population of glycosaminoglycans and the biological sample that provided the population of glycosaminoglycans.

[0003] Provided in certain embodiments herein is a process for diagnosing the identity and/or severity of abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., an MPS disorder or more specifically an MPS IV (Morquio syndrome or mucopolysaccharidosis type IV) disorder, the process comprising the steps of:

a. using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by:

    treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual to transform the glycosaminoglycans into the population of the one or more oligosaccharide;

b. displaying or recording the presence of or a measure of a population of one or more oligosaccharide.

[0004] In certain embodiments, provided is a process for diagnosing the presence, identity, and/or severity of abnormal degradation, biosynthesis and/or accumulation of

glycosaminoglycans in an individual, or a disorder thereof, e.g., MPS IV, the process comprising the steps of:

a. generating a biomarker comprising of one or more non-reducing end oligosaccharides, wherein the biomarker is a saturated oligosaccharide and is generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan enzymes (e.g., lyases), wherein prior to enzyme treatment, such oligosaccharide biomarker is not present in abundance in samples from individuals with abnormal glycosaminoglycan accumulation relative to individuals with normal glycosaminoglycan;
b. using an analytical instrument to detect the presence of and/or measure the amount of the biomarker produced and displaying or recording the presence of or a measure of a population of the biomarker.

[0005] In specific embodiments, the presence of and/or measure the amount of the biomarker is utilized to diagnose of the presence, identity, and/or severity of abnormal glycosaminoglycan accumulation. More specifically, in some embodiments, the presence of and/or measure the amount of the biomarker is utilized to diagnose of the presence, identity, and/or severity of MPS IV, including MPS IVA and MPS IVB.

[0006] In certain embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s).

[0007] In some embodiments, treating a population of glycosaminoglycans to transform the glycosaminoglycans into the population of the one or more oligosaccharide comprises contacting the glycosaminoglycans with at least one digesting glycosaminoglycan lyase. In some embodiments, the at least one digesting glycosaminoglycan lyase is one or more chondroitinase, one or more keratanase, or a combination thereof. In some embodiments, non-lyase enzymes can be used to digest glycosaminoglycans. In some embodiments, the enzyme is keratanase I or keratanase II. In other embodiments, the enzyme is chondroitinase, e.g., chondroitinase ABC, chondroitinase B, or chondroitinase AC.

[0008] In certain embodiments, the one or more oligosaccharides detected and/or measured are free of carbon-carbon unsaturation. In various embodiments, the abnormal glycosaminoglycan

accumulation comprises abnormal chondroitin sulfate accumulation, abnormal keratan sulfate accumulation, abnormal dermatan accumulation, or a combination thereof. In specific embodiments, the abnormal glycosaminoglycan accumulation is abnormal keratan sulfate accumulation. In specific embodiments, the abnormal glycosaminoglycan accumulation is abnormal chondroitin sulfate accumulation. In specific embodiments, the abnormal glycosaminoglycan accumulation is abnormal dermatan sulfate accumulation.

[0009] In various embodiments, any process described herein of preparing a transformed biological sample comprises purifying a population of oligosaccharides in the biological sample that has been treated with the at least one keratan sulfate, chondroitin sulfate, or dermatan sulfate digesting lyase, the transformed biological sample comprising the isolated population of oligosaccharides. In some embodiments, any process described herein of preparing a transformed biological sample comprises purifying a population of glycosaminoglycans in the biological sample prior to treatment with the at least one keratan sulfate, chondroitin sulfate, or dermatan sulfate digesting lyase.

[0010] In certain embodiments, any process described herein of detecting the presence of or measuring the amount of a population of one or more oligosaccharide present in a transformed biological sample comprises:

a. isolating a subpopulation of one or more oligosaccharides in the transformed biological sample; and
b. detecting the presence of and/or measuring the amount of one or more oligosaccharides present in the subpopulation.

[0011] In specific embodiments, a subpopulation of one or more oligosaccharides is isolated using, by way of non-limiting example, chromatography or electrophoresis. In specific embodiments, the chromatography is high performance liquid chromatography (HPLC), gas chromatography (GC), column chromatography, affinity chromatography, or thin layer chromatography (TLC). In some embodiments, any process of detecting oligosaccharides described herein comprises detecting oligosaccharides using mass spectrometry.

[0012] In some embodiments, any process described herein of preparing a transformed biological sample comprises tagging the reducing end of a representative portion of the one or more oligosaccharides in the transformed biological sample with a detectable label. In specific

embodiments, the detectable label is a mass label, a radio label, a fluorescent label, a chromophore label, or affinity label. In some embodiments, the tagged portion of the one or more oligosaccharides is detected or measured using UV-Vis spectroscopy, IR spectroscopy, mass spectrometry, or a combination thereof.

[0013] In certain embodiments, a digesting glycosaminoglycan lyase utilized in any process described herein comprises a chondroitin sulfate lyase (a lyase that digests chondroitin sulfate), a keratan sulfate lyase (a lyase that digests keratan sulfate), a dermatan sulfate lyase (a lyase that digests dermatan sulfate lyase), or a combination thereof. In other specific embodiments, a digesting glycosaminoglycan lyase utilized in any process described herein comprises keratan sulfate lyase, e.g., keratanase I or keratanase II. In other specific embodiments, a digesting glycosaminoglycan lyase utilized in any process described herein comprises chondroitin sulfate lyase, e.g., chondroitinase ABC. In other specific embodiments, a digesting glycosaminoglycan lyase utilized in any process described herein comprises dermatan sulfate lyase, e.g., chondroitinase B.

[0014] In some embodiments, the process provided herein comprises detecting or measuring an oligosaccharide of Formulas I-V or of any oligosaccharides shown in Figures 1-4.

[0015] In some embodiments, any process described herein comprises:

a. comparing an amount of a population of one or more oligosaccharide present in a transformed biological sample to an amount of a population of one or more oligosaccharide present in a control biological sample that has been treated in a manner substantially similar to the transformed biological sample.

[0016] In certain embodiments, a control biological sample utilized in any process described herein was provided from an individual that does not have mucopolysaccharidosis (e.g., a non-MPS cell line). In some embodiments, any control biological sample utilized in a process described herein was provided from an individual that has mucopolysaccharidosis. In specific embodiments, a control biological sample was provided from an individual that has MPS IV, e.g., MPS IVA, MPS IVB, or a combination thereof. In a specific embodiment, a control biological sample was provided from an individual that has MPS IVA. In a specific embodiment, a control biological sample was provided from an individual that has MPS IVB.

[0017] Provided in certain embodiments herein is an analytical sample comprising any oligosaccharide described herein, including an oligosaccharide described herein and further attached to a detectable label (e.g., at the reducing end of the oligosaccharide).

[0018] In specific embodiments, an analytical sample provided for herein is for use in high performance liquid chromatography. In some embodiments, an analytical sample provided for herein is for use in mass spectrometry. In certain embodiments, an analytical sample provided for herein is for use in gas chromatography. In some embodiments, any analytical sample provided herein comprises at least one monosaccharide, disaccharide or trisaccharide from a transformed biological sample from an individual with a disorder associated with abnormal glycosaminoglycan accumulation. In some embodiments, any analytical sample provided herein comprises at least one oligosaccharide consisting of more than three monosaccharide units (e.g., 4, 5, 6, 7, and 8) from a transformed biological sample from an individual with a disorder associated with abnormal glycosaminoglycan accumulation.

[0019] Provided in some embodiments herein is an analytical method comprising treating a biological sample that comprises glycosaminoglycans with at least one digesting glycosaminoglycan lyase to transform a representative portion of the glycosaminoglycans into one or more oligosaccharides. In certain embodiments, an analytical method provided for herein comprises purifying one or more oligosaccharides from other components of the biological sample. In some embodiments, the purifying step includes use of chromatography. In various embodiments, an analytical method provided for herein comprises detecting and/or measuring the presence of at least one of the oligosaccharides (e.g., after purification). In certain embodiments, oligosaccharides are detected and/or measured according to any process or method (used interchangeably herein) described herein using UV-Vis spectroscopy, IR spectroscopy, mass spectrometry, or a combination thereof. In some embodiments, any process described herein comprises tagging at least one of the oligosaccharides with a detectable label. In certain embodiments, the at least one digesting glycosaminoglycan lyase utilized in any process or method described herein comprises one or more chondroitinase (e.g., chondroitinase ABC, chondroitinase B, or chondroitinase AC), one or more keratanase (e.g., keratanase I or keratanase II), or a combination thereof.

[0020] In specific embodiments, an analytical method described herein is used in a method of detecting and/or measuring one or more oligosaccharides that are free of carbon-carbon unsaturation.

[0021] In certain specific embodiments, the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IV. As such, in some embodiments, provided herein is a process for diagnosing the presence, identity, and/or severity of MPS IV (e.g., MPS IVA or MPS IVB) in an individual, the process comprising the steps of:

a. generating one or more biomarkers comprising of one or more non-reducing end oligosaccharides, wherein the one or more biomarkers are oligosaccharides and are generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan enzymes, wherein after enzyme treatment, the amount of the one or more biomarkers in samples from individuals with abnormal glycosaminoglycan accumulation are different from the amount of the one or more biomarkers in samples from individuals with normal glycosaminoglycan, and

b. using an analytical instrument to detect the presence of and/or measure the amount of the one or more biomarkers produced and displaying or recording the presence of or a measure of a population of the one or more biomarkers;

wherein the presence of and/or measure the amount of the one or more biomarkers is utilized to determine the presence, identity, and/or severity of MPS IV.

[0022] In some embodiments, the process provided herein comprises generating a first biomarker and a second biomarker, and wherein the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is different from the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

[0023] In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is more than one fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-

fold to 100-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold to 20-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

[0024] In a specific embodiment, the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans provided herein is MPS IVA. In another specific embodiment, the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans provided herein is MPS IVB.

[0025] In some embodiments, the at least one digesting glycosaminoglycan enzymes is one or more chondroitin sulfate digesting lyase, one or more dermatan sulfate digesting lyase, one or more keratan sulfate digesting lyase, or a combination thereof. In some embodiments, the at least one digesting glycosaminoglycan enzymes provided herein are one or more chondroitinases, one or more keratanases, or a combination thereof. In some embodiments, the at least one digesting glycosaminoglycan enzymes comprise one or more keratanases. In a specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise keratanase I. In another specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise keratanase II. In yet another specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise chondroitinase ABC. In yet another specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise chondroitinase B. In yet another specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise chondroitinase AC.

[0026] In some embodiments, the abnormal glycosaminoglycan accumulation provided herein comprises abnormal chondroitin sulfate accumulation, abnormal keratan sulfate accumulation, abnormal dermatan sulfate accumulation, or a combination thereof. In some embodiments, the abnormal glycosaminoglycan accumulation is abnormal keratan sulfate accumulation. In some embodiments, the abnormal glycosaminoglycan accumulation is abnormal chondroitin sulfate accumulation. In some embodiments, the abnormal glycosaminoglycan accumulation is abnormal dermatan sulfate accumulation.

[0027] In some embodiments, the process provided herein further comprises purifying transformed biological sample using chromatography or electrophoresis. In some embodiments,

the chromatography provided herein is high performance liquid chromatography (HPLC), gas chromatography (GC), column chromatography, affinity chromatography, or thin layer chromatography (TLC).

[0028] In some embodiments, the oligosaccharides provided herein are detected using mass spectrometry. In some embodiments, the process of preparing transformed biological sample further comprises tagging the reducing end of a representative portion of the one or more oligosaccharides in the transformed biological sample with a detectable label. In some embodiments, the detectable label is a mass label, a radio label, a fluorescent label, a chromophore label, or affinity label. In some embodiments, the tagged portion of the one or more oligosaccharides is detected or measured using UV-Vis spectroscopy, IR spectroscopy, mass spectrometry, or a combination thereof.

[0029] In some embodiments of the various processes provided herein, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes comprises keratan sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprise keratanase I; wherein a first biomarker and a second biomarker are generated; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IVA. In some embodiments, the first biomarker is odd numbered oligosaccharide, and the second biomarker is even numbered oligosaccharide. In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is higher than the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is 2-fold to 20-fold of the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is 1:2; and the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA is 1:5. In some embodiments, the odd numbered

oligosaccharides are odd numbered oligosaccharides shown in Figure 2. In some embodiments, the old numbered oligosaccharides include oligosaccharides having three, five, or seven monosaccharide units.

[0030] In some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises keratan sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprise keratanase II; wherein a first biomarker and a second biomarker are generated; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IVA. In some embodiments, the first biomarker is oligosaccharides with sulfated galactose at the non-reducing end; and the second biomarker is oligosaccharides with unsulfated galactose at the non-reducing end. In some embodiments, the first biomarker and the second biomarker are tetrasaccharides. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is higher than the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 100-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is 10:1; and the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA is 1:1. In some embodiments, the oligosaccharides with sulfated galactose at the non-reducing end are those shown in Figure 3. In some embodiments, the oligosaccharides with unsulfated galactose at the non-reducing end are those shown in Figure 3.

[0031] In some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises chondroitin sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprise chondroitinase ABC; wherein a first biomarker and a second biomarker are generated; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IVA. In some embodiments, the first biomarker is oligosaccharide with 6-sulfated GalNAc at the non-reducing end; and the second biomarker is oligosaccharide with 4-sulfated GalNAc at the non-reducing end. In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is higher than the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 100-fold of the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is more than 10:1; and the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA is less than 1:1. In some embodiments, the oligosaccharides with 6-sulfated GalNAc at the non-reducing end is 6-sulfated GalNAc, and the oligosaccharides with 4-sulfated GalNAc at the non-reducing end is 4-sulfated GalNAc.

[0032] In some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes comprises chondroitin sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprises a chondroitinase (such as chondroitinase ABC, chondroitinase AC and chondroitinase B); wherein a biomarker is generated, the biomarker

being 6-sulfated GalNAc; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IVA.

[0033] In some embodiments, the method provided herein further comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is higher than a reference level of 6-sulfated GalNAc.

[0034] In some embodiments, the reference level of 6-sulfated GalNAc is determined by using a control sample obtained from a normal individual without MPS IVA. In other embodiments, the reference level of 6-sulfated GalNAc is determined by using a control sample obtained from the same individual, wherein the control sample is not digested with chondroitinase ABC.

[0035] In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is 2-fold to 1000-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 20-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 50-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 100-fold of the reference level of 6-sulfated GalNAc.

[0036] In some embodiments, the method provided herein further comprises administering a treatment agent to the individual diagnosed as having MPS IVA.

[0037] In certain embodiments, a process described herein includes a method of monitoring the treatment of disorders associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans (e.g. MPS IV), the methods comprising:

a. following administration of an agent for treating MPS (e.g., MPS IV) to an individual in need thereof, generating a biomarker comprising of one or more non-reducing end oligosaccharides, wherein the biomarker is a saturated oligosaccharide and is generated by treating a population of glycosaminoglycans,

in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase, wherein prior to lyase treatment, such oligosaccharide biomarker is not present in abundance in samples from individuals with abnormal glycosaminoglycan accumulation relative to individuals with normal glycosaminoglycan;

b. using an analytical instrument to detect the presence of and/or measure the amount of the biomarker produced and displaying or recording the presence of or a measure of a population of the biomarker.

[0038] In specific embodiments, increases or decreases in the amount of the biomarker measured (e.g., as compared to a biological sample previously analyzed in a similar or identical manner) is utilized to monitor the treatment of disorders associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans.

[0039] In some specific embodiments, provided herein is a process of monitoring the efficacy of a treatment to an individual with MPS IVA, comprising:

a. administering a treatment to the individual with MPS IVA;

b. obtaining a biological sample from the individual with MPS IVA;

c. treating a population of chondroitin sulfates or dermatan sulfates, in or isolated from the biological sample, with chondroitinase ABC;

d. using an analytical instrument to measure the amount of 6-sulfated GalNAc produced and displaying or recording a measure of 6-sulfated GalNAc;

e. comparing the level of 6-sulfated GalNAc in the biological sample with a reference level of 6-sulfated GalNAc; and

f. determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than the reference level of 6-sulfated GalNAc,

wherein the reference level of the biomarker is determined by using a control sample obtained from the same individual prior to administering the treatment agent.

[0040] In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 90% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided

herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 80% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 50% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 30% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 20% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 5% of the reference level of 6-sulfated GalNAc.

[0041] In some embodiments, the treatment comprises administering elosulfase alfa. In some embodiments, the treatment is selected from a group consisting of enzyme replacement therapy (ERT), bone marrow transplantation (BMT), and umbilical cord blood transplantation (UCBT).

[0042] In some embodiments of the various processes provided herein, the oligosaccharide provided herein is selected from a group consisting of monosaccharide, disaccharide, and trisaccharide. In a specific embodiment, the oligosaccharide provided herein is a monosaccharide. In other embodiments, the oligosaccharide provided herein comprises more than three monosaccharides.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0044] **Figure 1** illustrates digestions by keratanase I and keratanase II.

[0045] **Figures 2A and 2B** illustrate analysis of keratan sulfate using keratanase I.

[0046] **Figures 3A and 3B** illustrate analysis of keratan sulfate using keratanase II.

[0047] **Figures 4A and 4B** illustrate analysis of chondroitin sulfate using chondroitinase ABC.

[0048] **Figure 5** shows the levels of 6-sulfated GalNAc in MPS IVA patients and normal individuals after digestion with chondroitinase ABC.

[0049] **Figure 6** shows the levels of 6-sulfated GalNAc (after digestion with chondroitinase ABC) before and after treatment with VIMIZIM® in adult MPS IVA patients.

DETAILED DESCRIPTION OF THE INVENTION

[0050] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[0051] Provided in certain embodiments herein are analytical methods for detecting and/or identifying glycosaminoglycans (GAGs) or other glycans (e.g., glycolipids) in biological sample. In certain embodiments, the glycans, e.g., glycosaminoglycans, are present in cells within a biological sample (e.g., within a lysosome thereof), and/or are present in a biological sample free of cells. In certain embodiments, provided herein is a method of diagnosing any disorder characterized by the accumulation of glycosaminoglycans, such as a lysosomal storage disease (LSD). In some embodiments, the glycosaminoglycan accumulation is a primary accumulative effect. In certain instances, primary accumulative effects include accumulation that is a direct result of an abnormal biosynthetic process, such as abnormal production enzymes involved in the glycan biosynthetic pathway (e.g., under-production or production of poorly functioning enzymes), including glycan bio-synthesis or depolymerization. In other embodiments, the glycosaminoglycan accumulation is a secondary accumulative effect. In certain embodiments, a secondary accumulative effect results from a cascading effect, e.g., accumulation of other components, such as glycosaminoglycans or other glycans, such as glycolipids, causes the glycosaminoglycans biosynthetic pathway to be hindered or interrupted.

[0052] In certain embodiments, glycosaminoglycans include, by way of non-limiting example, chondroitin sulfate, dermatan sulfate, keratan sulfate, or the like, or a combination thereof. In certain embodiments, an analytical method provided herein comprising treating a biological

sample that comprises glycosaminoglycans with at least one agent suitable for cleaving bonds between saccharide residues of glycosaminoglycans. In specific embodiments, treating a biological sample that comprises glycosaminoglycans with at least one agent suitable for cleaving bonds between saccharide residues of glycosaminoglycans comprises treating the biological sample with one or more digesting glycosaminoglycan lyase. In some embodiments, any glycosaminoglycan lyase suitable for cleaving the bonds (e.g., the bonds linking saccharide residues of the glycosaminoglycans to one another) of a glycosaminoglycan analyze is utilized. In some embodiments, the lyase is utilized to transform a representative portion of the glycosaminoglycans into one or more oligosaccharides. In certain embodiments, such glycosaminoglycan lyases are suitable for preparing mono-, di- and/or tri-saccharides from the glycosaminoglycan present. Glycosaminoglycan lyases suitable for use in various embodiments provided herein include, by way of non-limiting example, one or more chondroitinase (e.g., chondroitinase ABC, chondroitinase B, and chondroitinase AC), one or more keratanase, or a combination thereof. Other glycans that are optionally detected by a method described herein include, e.g., glycolipids.

[0053] In some embodiments of the various processes provided herein, the glycosaminoglycan digesting enzyme is a lyase. In other embodiments of the various processes provided herein, the glycosaminoglycan digesting enzyme is a non-lyase.

[0054] In some embodiments, lyases utilized herein include, by way of non-limiting example, Hyaluronate lyase, Pectate lyase, Poly(beta-D-mannuronate) lyase, Chondroitin ABC lyase, Chondroitin AC lyase, Oligogalacturonide lyase, Heparin lyase, Heparin-sulfate lyase, Pectate disaccharide-lyase, Pectin lyase, Poly(alpha-L-guluronate) lyase, Xanthan lyase, Exo-(1->4)-alpha-D-glucan lyase, Glucuronan lyase, Anhydrosialidase, Levan fructotransferase, Inulin fructotransferase, Inulin fructotransferase, Chondroitin B lyase. In certain instances, Hyaluronate lyase (EC 4.2.2.1) is an enzyme that catalyzes the cleavage or hyaluronate chains at a beta-D-GalNAc-(1->4)-beta-D-GlcA bond, ultimately breaking the polysaccharide down to 3-(4-deoxy-beta-D-gluc-4-enuronosyl)-N-acetyl-D-glucosamine. In some instances, Pectate lyase (EC 4.2.2.2) is an enzyme that catalyzes the eliminative cleavage of (1->4)-alpha-D-galacturonan to give oligosaccharides with 4-deoxy-alpha-D-galact-4-enuronosyl groups at their non-reducing ends. In certain instances, Poly(beta-D-mannuronate) lyase (EC 4.2.2.3) is an enzyme that

catalyzes the eliminative cleavage of polysaccharides containing beta-D-mannuronate residues to give oligosaccharides with 4-deoxy-alpha-L-erythro-hex-4-enopyranuronosyl groups at their ends. In some instances, Chondroitin ABC lyase (EC 4.2.2.4) is an enzyme that catalyzes the eliminative degradation of polysaccharides containing 1,4-beta-D-hexosaminyl and 1,3-beta-D-glucuronosyl linkages to disaccharides containing 4-deoxy-beta-D-gluc-4-enuronosyl groups. In some instances, Chondroitin ABC lyase (EC 4.2.2.4) also catalyzes the eliminative cleavage of dermatan sulfate containing 1,4-beta-D-hexosaminyl and 1,3-beta-D-glucurosonyl or 1,3-alpha-L-iduronosyl linkages to disaccharides containing 4-deoxy-beta-D-gluc-4-enuronosyl groups to yield a 4,5-unsaturated dermatan-sulfate disaccharide (deltaUA-GalNAc-4S). In certain instances, Chondroitin AC lyase (EC 4.2.2.5) is an enzyme that catalyzes the eliminative degradation of polysaccharides containing 1,4-beta-D-hexosaminyl and 1,3-beta-D-glucuronosyl linkages to disaccharides containing 4-deoxy-beta-D-gluc-4-enuronosyl groups. In some instances, Oligogalacturonide lyase (EC 4.2.2.6) is an enzyme that catalyzes the cleavage of 4-(4-deoxy-beta-D-gluc-4-enuronosyl)-D-galacturonate into 2 5-dehydro-4-deoxy-D-glucuronate. In certain instances, Heparin lyase (EC 4.2.2.7) is an enzyme that catalyzes the eliminative cleavage of polysaccharides containing 1,4-linked D-glucuronate or L-iduronate residues and 1,4-alpha-linked 2-sulfoamino-2-deoxy-6-sulfo-D-glucose residues to give oligosaccharides with terminal 4-deoxy-alpha-D-gluc-4-enuronosyl groups at their non-reducing ends. In some instances, Heparin lyase (EC 4.2.2.7) tolerates alternative sulfation of the substrate. In some instances, Heparin-sulfate lyase (EC 4.2.2.8) is an enzyme that catalyzes the eliminative cleavage of polysaccharides containing 1,4-linked D-glucuronate or L-iduronate residues and 1,4-alpha-linked 2-sulfoamino-2-deoxy-6-sulfo-D-glucose residues to give oligosaccharides with terminal 4-deoxy-alpha-D-gluc-4-enuronosyl groups at their non-reducing ends. In some instances, Heparin-sulfate lyase (EC 4.2.2.8) tolerates alternative sulfation of the substrate. In certain instances, Pectate disaccharide-lyase (EC 4.2.2.9) is an enzyme that catalyzes the eliminative cleavage of 4-(4-deoxy-alpha-D-galact-4-enuronosyl)-D-galacturonate from the reducing end of pectate, i.e. de-esterified pectin. In some instances, Pectin lyase (EC 4.2.2.10) is an enzyme that catalyzes the eliminative cleavage of (1->4)-alpha-D-galacturonan methyl ester to give oligosaccharides with 4-deoxy-6-O-methyl-alpha-D-galact-4-enuronosyl groups at their non-reducing ends. In certain instances, Poly(alpha-L-guluronate) lyase (EC 4.2.2.11) is an enzyme

that catalyzes the eliminative cleavage of polysaccharides containing a terminal alpha-L-guluronate group, to give oligosaccharides with 4-deoxy-alpha-L-erythro-hex-4-enuronosyl groups at their non-reducing ends. In some instances, Xanthan lyase (EC 4.2.2.12) is an enzyme that catalyzes the cleavage of the beta-D-mannosyl-beta-D-1,4-glucuronosyl bond on the polysaccharide xanthan. In certain instances, Exo-(1->4)-alpha-D-glucan lyase (E.C. 4.2.2.13) is an enzyme that catalyzes the sequential degradation of (1->4)-alpha-D-glucans from the non-reducing end with the release of 1,5-anhydro-D-fructose. In some instances, Glucuronan lyase (EC 4.2.2.14) is an enzyme that catalyzes the eliminative cleavage of (1->4)-beta-D-glucuronans. This produces either oligosaccharides with 4-deoxy-beta-D-gluc-4-enuronosyl groups at their non-reducing ends, or, if the substrate is completely degraded, glucuronans produce tetrasaccharides. In certain instances, Anhydrosialidase (EC 4.2.2.15) is an enzyme that catalyzes the elimination of alpha-sialyl groups in N-acetylneuraminic acid glycosides, releasing 2,7-anhydro-alpha-N-acetylneuraminate. In some instances, Levan fructotransferase (DFA-IV-forming) (EC 4.2.2.16) is an enzyme that produces di-beta-D-fructofuranose 2,6':2',6-dianhydride (DFA IV) by successively eliminating the diminishing (2->6)-beta-D-fructan (levan) chain from the terminal D-fructosyl-D-fructosyl disaccharide. In certain instances, Inulin fructotransferase (DFA-I-forming) (EC 4.2.2.17) is an enzyme that produces alpha-D-fructofuranose beta-D-fructofuranose 1,2':2,1'-dianhydride (DFA I) by successively eliminating the diminishing (2->1)-beta-D-fructan (inulin) chain from the terminal D-fructosyl-D-fructosyl disaccharide. In some instances, Inulin fructotransferase (DFA-III-forming) (EC 4.2.2.18) is an enzyme that produces alpha-D-fructofuranose beta-D-fructofuranose 1,2':2,3'-dianhydride (DFA III) by successively eliminating the diminishing (2->1)-beta-D-fructan (inulin) chain from the terminal D-fructosyl-D-fructosyl disaccharide. In certain instances, Chondroitin B lyase (EC 4.2.2.19) is an enzyme that catalyzes the eliminative cleavage of dermatan sulfate containing 1,4-beta-D-hexosaminyl and 1,3-beta-D-glucurosonyl or 1,3-alpha-L-iduronosyl linkages to disaccharides containing 4-deoxy-beta-D-gluc-4-enuronosyl groups to yield a 4,5-unsaturated dermatan-sulfate disaccharide (deltaUA-GalNAc-4S). Any other suitable enzyme is also optionally utilized. For example, any keratanase may be used, e.g., as isolated from bacteria or evolved/designed from a related lyase.

[0055] In some embodiments of the various processes described herein, the enzymes provided herein include, by way of non- limiting example, a glycosidase. Non- limiting examples of glycosidase that are optionally utilized in the processes described herein include, by way of non-limiting example, enzymes categorized as 3.2.1. X by BRENDA (the comprehensive Enzyme Information System) including 3.2.1.1 alpha-amylase, 3.2.1. Bl extracellular agarase, 3.2.1.2 beta-amylase, 3.2.1.3 glucan 1,4-alpha-glucosidase, 3.2.1.4 cellulase, 3.2.1.5 licheninase, 3.2.1.6 endo-l,3(4)-beta-glucanase, 3.2.1.7 inulinase, 3.2.1.8 endo-1,4- beta-xylanase, 3.2.1.9 amylopectin-l,6-glucosidase, 3.2.1.10 oligo-l,6-glucosidase, 3.2.1.11 dextranase, 3.2.1.12 cycloheptaglucanase, 3.2.1.13 cyclohexaglucanase, 3.2.1.14 chitinase, 3.2.1.15 polygalacturonase, 3.2.1.16 alginase, 3.2.1.17 lysozyme, 3.2.1.18 exo-alpha- sialidase, 3.2.1.19 heparinase, 3.2.1.20 alpha-glucosidase, 3.2.1.21 beta-glucosidase, 3.2.1.22 alpha-galactosidase, 3.2.1.23 beta-galactosidase, 3.2.1.24 alpha-mannosidase, 3.2.1.25 beta-mannosidase, 3.2.1.26 beta-fructofuranosidase, 3.2.1.27 alpha-1,3- glucosidase, 3.2.1.28 alpha, alpha-trehalase, 3.2.1.29 chitobiase, 3.2.1.30 beta-D- acetylglucosaminidase, 3.2.1.31 beta-glucuronidase, 3.2.1.32 xylan endo-l,3-beta- xylosidase, 3.2.1.33 amylo-alpha-l,6-glucosidase, 3.2.1.34 chondroitinase, 3.2.1.35 hyaluronoglucosaminidase, 3.2.1.36 hyaluronoglucuronidase, 3.2.1.37 xylan 1,4-beta-xylosidase, 3.2.1.38 beta-D-fucosidase, 3.2.1.39 glucan endo-l,3-beta-D-glucosidase, 3.2.1.40 alpha-L-rhamnosidase, 3.2.1.41 pullulanase, 3.2.1.42 GDP-glucosidase, 3.2.1.43 beta-L-rhamnosidase, 3.2.1.44 fucoidanase, 3.2.1.45 glucosylceramidase, 3.2.1.46 galactosylceramidase, 3.2.1.47 galactosylgalactosylglucosylceramidase, 3.2.1.48 sucrose alpha-glucosidase, 3.2.1.49 alpha-N-acetylgalactosaminidase, 3.2.1.50 alpha-N- acetylglucosaminidase, 3.2.1.51 alpha-L-fucosidase, 3.2.1.52 beta-N-acetylhexosaminidase, 3.2.1.53 beta-N-acetylgalactosaminidase, 3.2.1.54 cyclomaltodextrinase, 3.2.1.55 alpha-N- arabinofuranosidase, 3.2.1.56 glucuronosyl-disulfoglucosamine glucuronidase, 3.2.1.57 isopullulanase, 3.2.1.58 glucan 1,3-beta-glucosidase, 3.2.1.59 glucan endo-l,3-alpha- glucosidase, 3.2.1.60 glucan 1,4-alpha-maltotetraohydrolase, 3.2.1.61 mycodextranase, 3.2.1.62 glycosylceramidase, 3.2.1.63 1,2-alpha-L-fucosidase, 3.2.1.64 2,6-beta-fructan 6- levanbiohydrolase, 3.2.1.65 levanase, 3.2.1.66 quercitrinase, 3.2.1.67 galacturan 1,4-alpha- galacturonidase, 3.2.1.68 isoamylase, 3.2.1.69 amylopectin 6-glucanohydrolase, 3.2.1.70 glucan 1,6-alpha-glucosidase, 3.2.1.71 glucan endo-l,2-beta-glucosidase, 3.2.1.72 xylan 1,3-beta-xylosidase, 3.2.1.73 licheninase, 3.2.1.74 glucan 1,4-beta-

glucosidase, 3.2.1.75 glucan endo-l,6-beta-glucosidase, 3.2.1.76 L-iduronidase, 3.2.1.77 mannan l,2-(l,3)-alpha- mannosidase, 3.2.1.78 mannan endo-l,4-beta-mannosidase, 3.2.1.79 alpha-L-arabinofuranoside hydrolase, 3.2.1.80 fructan beta-fructosidase, 3.2.1.81 beta-agarase, 3.2.1.82 exo-poly-alpha-galacturonosidase, 3.2.1.83 kappa-carrageenase, 3.2.1.84 glucan 1,3-alpha-glucosidase, 3.2.1.85 6-phospho-beta-galactosidase, 3.2.1.86 6-phospho-beta- glucosidase, 3.2.1.87 capsular-polysaccharide endo-l,3-alpha-galactosidase, 3.2.1.88 beta- L-arabinosidase, 3.2.1.89 arabinogalactan endo-l,4-beta-galactosidase, 3.2.1.90 arabinogalactan endo- 1,3 -beta-galactosidase, 3.2.1.91 cellulose 1,4-beta-cellobiosidase, 3.2.1.92 peptidoglycan beta-N-acetylmuramidase, 3.2.1.93 alpha,alpha-phosphotrehalase, 3.2.1.94 glucan 1,6-alpha-isomaltosidase, 3.2.1.95 dextran 1,6-alpha-isomaltotriosidase, 3.2.1.96 mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase, 3.2.1.97 glycopeptide alpha-N-acetylgalactosaminidase, 3.2.1.98 glucan 1,4-alpha-maltohexaosidase, 3.2.1.99 arabinan endo-l,5-alpha-L-arabinosidase, 3.2.1.100 mannan 1,4-mannobiosidase, 3.2.1.101 mannan endo-l,6-alpha-mannosidase, 3.2.1.102 blood-group-substance endo-l,4-beta- galactosidase, 3.2.1.103 keratan-sulfate endo-l,4-beta-galactosidase, 3.2.1.104 steryl-beta- glucosidase, 3.2.1.105 3alpha(S)-strictosidine beta-glucosidase, 3.2.1.106 mannosyl- oligosaccharide glucosidase, 3.2.1.107 protein-glucosylgalactosylhydroxylysine glucosidase, 3.2.1.108 lactase, 3.2.1.109 endogalactosaminidase, 3.2.1.110 mucinaminylserine mucinaminidase, 3.2.1.111 1,3-alpha-L-fucosidase, 3.2.1.112 2- deoxyglucosidase, 3.2.1.113 mannosyl-oligosaccharide 1,2-alpha-mannosidase, 3.2.1.114 mannosyl-oligosaccharide 1,3-1,6-alpha-mannosidase, 3.2.1.115 branched-dextran exo-1,2- alpha-glucosidase, 3.2.1.116 glucan 1,4-alpha-maltotriohydrolase, 3.2.1.117 amygdalin beta-glucosidase, 3.2.1.118 prunasin beta-glucosidase, 3.2.1.119 vicianin beta-glucosidase, 3.2.1.120 oligoxyloglucan beta-glycosidase, 3.2.1.121 polymannuronate hydrolase, 3.2.1.122 maltose-6'-phosphate glucosidase, 3.2.1.123 endoglycosylceramidase, 3.2.1.124 3-deoxy-2-octulosonidase, 3.2.1.125 raucaffricine beta-glucosidase, 3.2.1.126 coniferin beta-glucosidase, 3.2.1.127 1,6-alpha-L-fucosidase, 3.2.1.128 glycyrrhizinate beta-glucuronidase, 3.2.1.129 endo-alpha-sialidase, 3.2.1.130 glycoprotein endo-alpha-1,2-mannosidase, 3.2.1.131 xylan alpha- 1,2-glucuronosidase, 3.2.1.132 chitosanase, 3.2.1.133 glucan 1,4-alpha-maltohydrolase, 3.2.1.134 difructose-anhydride synthase, 3.2.1.135 neopullulanase, 3.2.1.136 glucuronoarabinoxylan endo-l,4-beta-xylanase, 3.2.1.137 mannan exo-

l,2-l,6-alpha-mannosidase, 3.2.1.138 anhydrosialidase, 3.2.1.139 alpha- glucuronidase, 3.2.1.140 lacto-N-biosidase, 3.2.1.141 4-alpha-D-{(l->4)-alpha-D- glucano} trehalose trehalohydrolase, 3.2.1.142 limit dextrinase, 3.2.1.143 poly(ADP-ribose) glycohydrolase, 3.2.1.144 3-deoxyoctulosonase, 3.2.1.145 galactan 1,3-beta-galactosidase, 3.2.1.146 beta-galactofuranosidase, 3.2.1.147 thioglucosidase, 3.2.1.148 ribosylhomocysteinase, 3.2.1.149 beta-primeverosidase, 3.2.1.150 oligoxyloglucan reducing-end-specific cellobiohydrolase, 3.2.1.151 xyloglucan- specific endo-beta-1,4- glucanase, 3.2.1.152 mannosylglycoprotein endo-beta-mannosidase, 3.2.1.153 fructan beta- (2,l)-fructosidase, 3.2.1.154 fructan beta-(2,6)-fructosidase, 3.2.1.155 xyloglucan-specific exo-beta- 1, 4-glucanase, 3.2.1.156 oligosaccharide reducing-end xylanase, 3.2.1.157 iota- carrageenase 3.2.1.158 alpha-agarase, 3.2.1.159 alpha-neoagaro-oligosaccharide hydrolase, 3.2.1.160 xyloglucan-specific exo-beta- 1, 4-glucanase, 3.2.1.161 beta-apiosyl-beta- glucosidase, 3.2.1.162 lambda-carrageenase, 3.2.1.163 1,6-alpha-D-mannosidase, 3.2.1.164 galactan endo-l,6-beta-galactosidase, 3.2.1.165 exo-l,4-beta-D-glucosaminidase, or a combination thereof.

[0056] In other embodiments of the various processes described herein, the enzymes provided herein include, by way of non- limiting example, a sulfatase including, e.g., enzymes categorized as 3.1.6.X by BRENDA (the comprehensive Enzyme Information System) including 3.1.6.1 arylsulfatase, 3.1.6.2 steryl-sulfatase, 3.1.6.3 glycosulfatase, 3.1.6.4 N- acetylgalactosamine-6-sulfatase, 3.1.6.5 sinigrin sulfohydrolase; myrosulfatase, 3.1.6.6 choline-sulfatase, 3.1.6.7 cellulose-polysulfatase, 3.1.6.8 cerebroside-sulfatase, 3.1.6.9 chondro-4-sulfatase, 3.1.6.10 chondro-6-sulfatase, 3.1.6.11 disulfoglucosamine-6-sulfatase, 3.1.6.12 N-acetylgalactosamine-4-sulfatase, 3.1.6.13 iduronate-2-sulfatase, 3.1.6.14 N- acetylglucosamine-6-sulfatase, 3.1.6.15 N-sulfoglucosamine-3-sulfatase, 3.1.6.16 monomethyl-sulfatase, 3.1.6.17 D-lactate-2-sulfatase, 3.1.6.18 glucuronate-2-sulfatase, 3.10.1.1 N-sulfoglucosamine sulfohydrolase, or combinations thereof.

[0057] In yet other embodiments of the various processes described herein, the enzymes provided herein include, by way of non- limiting example, a deacetylase, e.g., an exo-deacetylase, including, by way of non- limiting example, the alpha-glucosaminide N-acetyltransferase (2.3.1.78) or similar enzymes.

[0058] In yet other embodiments of the various processes described herein, the enzymes provided herein include, by way of non- limiting example, a carbohydrate phosphatase including, e.g., 3.1.3.1 alkaline phosphatase, 3.1.3.2 acid phosphatase, 3.1.3.B2 diacylglycerol pyrophosphate phosphatase, 3.1.3.3 phosphoserine phosphatase, 3.1.3.4 phosphatidate phosphatase, 3.1.3.5 5 '-nucleotidase, 3.1.3.6 3 '-nucleotidase, 3.1.3.7 3'(2'),5'-bisphosphate nucleotidase, 3.1.3.8 3-phytase, 3.1.3.9 glucose-6-phosphatase, 3.1.3.10 glucose-1- phosphatase, 3.1.3.11 fructose-bisphosphatase, 3.1.3.12 trehalose-phosphatase, 3.1.3.13 bisphosphoglycerate phosphatase, 3.1.3.14 methylphosphothioglycerate phosphatase, 3.1.3.15 histidinol-phosphatase, 3.1.3.16 phosphoprotein phosphatase, 3.1.3.17 [phosphorylase] phosphatase, 3.1.3.18 phosphoglycolate phosphatase, 3.1.3.19 glycerols- phosphatase, 3.1.3.20 phosphoglycerate phosphatase, 3.1.3.21 glycerol- 1 -phosphatase, 3.1.3.22 mannitol-1 -phosphatase, 3.1.3.23 sugar-phosphatase, 3.1.3.24 sucrose-phosphate phosphatase, 3.1.3.25 inositol-phosphate phosphatase, 3.1.3.26 4-phytase, 3.1.3.27 phosphatidylglycerophosphatase, 3.1.3.28 ADP-phosphoglycerate phosphatase, 3.1.3.29 N- acylneuraminate-9-phosphatase, 3.1.3.30 3'-phosphoadenylylsulfate 3 '-phosphatase, 3.1.3.31 nucleotidase, 3.1.3.32 polynucleotide 3 '-phosphatase, 3.1.3.33 polynucleotide 5'- phosphatase, 3.1.3.34 deoxynucleotide 3'-phosphatase, 3.1.3.35 thymidylate 5 '-phosphatase, 3.1.3.36 phosphoinositide 5 -phosphatase, 3.1.3.37 sedoheptulose-bisphosphatase, 3.1.3.38 3 -phosphoglycerate phosphatase, 3.1.3.39 streptomycin-6-phosphatase, 3.1.3.40 guanidinodeoxy-scyllo-inositol-4-phosphatase, 3.1.3.41 4-nitrophenylphosphatase, 3.1.3.42 [glycogen-synthase-D] phosphatase, 3.1.3.43 [pyruvate dehydrogenase (acetyl- transferring)]-phosphatase, 3.1.3.44 [acetyl-CoA carboxylase]-phosphatase, 3.1.3.45 3- deoxy-manno-octulosonate-8-phosphatase, 3.1.3.46 fructose-2,6-bisphosphate 2- phosphatase, 3.1.3.47 [hydroxymethylglutaryl-CoA reductase (NADPH)]-phosphatase, 3.1.3.48 protein-tyrosine-phosphatase, 3.1.3.49 [pyruvate kinase] -phosphatase, 3.1.3.50 sorbitol-6-phosphatase, 3.1.3.51 dolichyl-phosphatase, 3.1.3.52 [3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)]-phosphatase, 3.1.3.53 [myosin-light- chain] phosphatase, 3.1.3.54 fructose-2,6-bisphosphate 6-phosphatase, 3.1.3.55 caldesmon-phosphatase, 3.1.3.56 inositol-polyphosphate 5-phosphatase, 3.1.3.57 inositol- 1,4- bisphosphate 1 -phosphatase, 3.1.3.58 sugar-terminal-phosphatase, 3.1.3.59 alkylacetylglycerophosphatase, 3.1.3.60 phosphoenolpyruvate phosphatase, 3.1.3.61 inositol- 1, 4, 5-trisphosphate 1 -

phosphatase, 3.1.3.62 multiple inositol-polyphosphate phosphatase, 3.1.3.63 2-carboxy-D-arabinitol- 1 -phosphatase, 3.1.3.64 phosphatidylinositol- 3 -phosphatase, 3.1.3.65 inositol- 1, 3 -bisphosphate 3 -phosphatase, 3.1.3.66 phosphatidylinositol-3,4-bisphosphate 4-phosphatase, 3.1.3.67 phosphatidylinositol-3,4,5- trisphosphate 3 -phosphatase, 3.1.3.68 2-deoxyglucose-6-phosphatase, 3.1.3.69 glucosylglycerol3 -phosphatase, 3.1.3.70 mannosyl-3-phosphoglycerate phosphatase, 3.1.3.71 2-phosphosulfolactate phosphatase, 3.1.3.72 5-phytase, 3.1.3.73 alpha-ribazole phosphatase, 3.1.3.74 pyridoxal phosphatase, 3.1.3.75 phosphoethanolamine/phosphocholine phosphatase, 3.1.3.76 lipid-phosphate phosphatase, 3.1.3.77 acireductone synthase, 3.1.3.78 phosphatidylinositol-4,5-bisphosphate A- phosphatase, or 3.1.3.79 mannosylfructose-phosphate phosphatase, or a combination thereof.

[0059] In some embodiments, the analytical process comprises detecting and/or measuring the one or more oligosaccharide present in the biological sample after it has been treated with one or more glycosaminoglycan lyase. In some embodiments, the one or more oligosaccharide detected and/or measured is one or more monosaccharide, one or more disaccharide and/or one or more trisaccharide. In certain embodiments, the one or more oligosaccharides detected and/or measured (e.g., one or more monosaccharide, one or more disaccharide and/or one or more trisaccharide) are saturated at 4 and 5 carbons of the non-reducing end saccharide residue. In some embodiments, the non-reducing end residue of the one or more oligosaccharides detected and/or measured (e.g., one or more monosaccharide, one or more disaccharide and/or one or more trisaccharide) are free of carbon-carbon unsaturation. In certain embodiments, the one or more oligosaccharides detected and/or measured (e.g., one or more monosaccharide, one or more disaccharide and/or one or more trisaccharide) are free of carbon-carbon unsaturation. Biological samples suitable for analysis according to the methods and processes described herein include, by way of non-limiting example, blood, peripheral blood mononuclear cell (PBMC), serum, urine, hair, saliva, skin, tissue, plasma, cerebrospinal fluid (CSF), amniotic fluid, nipple aspirate, sputum, feces, synovial fluid, nails, or the like. In specific embodiments, the biological samples suitable for analysis according to the methods and processes described herein include, by way of non-limiting example, urine, serum, plasma, PBMC, or CSF. In certain embodiments, processes for detecting glycosaminoglycans in a sample comprise providing, from the individual, a test biological sample that comprises glycosaminoglycans. In some embodiments, providing a test

biological sample from an individual includes obtaining the sample from the individual or obtaining the sample from another source (e.g., from a technician or institution that obtained the sample from the individual). In some embodiments, the biological sample is obtained from any suitable source, e.g., any tissue or cell (e.g., urine, serum, plasma, PBMC or CSF) of an individual. In certain embodiments, the tissue and/or cell from which the glycosaminoglycans are recovered is obtained from liver tissue or cells, brain tissue or cells, kidney tissue or cells, or the like.

[0060] In certain embodiments, analytical methods provided herein further comprise methods of purification. In certain embodiments, purification methods are performed prior to treating a biological sample with a lyase, as described herein. In some embodiments, purification methods are performed after treating a biological sample with a lyase, as described herein. In certain embodiments, purification methods are utilized before and after treating a biological sample with a lyase, as described herein. In some embodiments, purification methods include purifying one or more glycosaminoglycan and/or one or more oligosaccharide from other components (e.g., cells, cell parts, other polysaccharides, or the like) of the biological sample. In certain embodiments, purification methods include purifying one or more glycosaminoglycan from other polysaccharides (e.g., other glycans, other glycosaminoglycans, other sugars, or the like).

[0061] In certain instances the glycosaminoglycans provided in a biological sample are present in lysosomes of cells. In some embodiments, any process described herein includes lysing a biological sample to free the glycosaminoglycans from the cells therein.

Diagnostics

[0062] Provided in some embodiments herein is a process for diagnosing the identity and/or severity of abnormal glycosaminoglycan (or other glycan, e.g., glycolipid) accumulation in an individual, or a disorder thereof, e.g., MPS IV, the process comprising the step of: detecting the presence of and/or measuring the amount of a population of one or more oligosaccharides present in a transformed biological sample (e.g., urine, serum, plasma, PBMC or CSF). In certain embodiments, the process for diagnosing the identity and/or severity of abnormal glycosaminoglycan accumulation in an individual is a process of diagnosing the individual as an individual suffering from, homozygous for, or symptomatic for such a disorder. In other embodiments, the process for diagnosing the identity and/or severity of abnormal

glycosaminoglycan accumulation in an individual (e.g., MPS IV) is a process of diagnosing the individual as an individual suffering from such a disorder as a carrier for, or heterozygous for, such a disorder. In some embodiments, individuals that are carriers for, or heterozygous for, such a disorder has an elevated level of glycosaminoglycan accumulation (e.g., when compared to a normal individual), but the elevated level is less than an individual diagnosed with having the disorder. In certain embodiments, individuals that are carriers for, or heterozygous for, such a disorder has an elevated level of glycosaminoglycan accumulation (e.g., when compared to a normal individual), but are asymptomatic (including substantially asymptomatic) for a glycosaminoglycan accumulation disorder. Carriers and individuals having a glycosaminoglycan accumulation disease are identified utilizing any appropriate procedure. For example, in certain embodiments, carriers or carrier specimens may be identified as accumulating, e.g., 2-100 times more glycosaminoglycan than a non-carrier or wild type specimen. Similarly, in some exemplary embodiments, individuals that are symptomatic or have a glycosaminoglycan accumulation disease state accumulate more than 2 times more (e.g., 2-100x) glycosaminoglycan than a carrier. In some embodiments, diagnosis of one or more carrier parent is optionally utilized to make a progeny risk assessment (e.g., likelihood of a child being a carrier for or having a disease state).

[0063] In some embodiments, provided herein is a process for diagnosing abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., MPS IV, the process comprising the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans (e.g., keratan sulfate, chondroitin sulfate, and dermatan sulfate), in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s).

[0064] In some embodiments, provided herein is a process for diagnosing the identity (or type, *e.g.,* keratan sulfate, chondroitin sulfate, dermatan sulfate or any other glycosaminoglycan) of abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., MPS IV,

the process comprising the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s).

[0065] In some embodiments, provided herein is a process for diagnosing the severity of abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., MPS IV, the process comprising the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s).

[0066] In some embodiments, provided herein is a process for diagnosing an individual as being a carrier of a gene that causes abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., MPS IV, the process comprising the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In certain instances, such a process involves determining the severity of abnormal glycosaminoglycan accumulation, wherein such accumulation is below a certain threshold (e.g., a predetermined level, a level whereby the individual becomes symptomatic, or the like). In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s).

[0067] In some embodiments, provided herein is a process for diagnosing abnormal glycosaminoglycan accumulation in a human infant (e.g., a newborn) or fetus, or a disorder

thereof, e.g., MPS IV, the process comprising the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s).

[0068] In further embodiments, any of the processes described herein further comprise the step of displaying or recording the presence of or a measure of a population of one or more oligosaccharide. The display may be on a computer screen or a paper print out. The recording may be on any computer readable disk (e.g., a hard drive, CD, DVD, portable memory device, such as a CF device or SD device, or the like), a sheet of paper, or the like.

[0069] In some embodiments, the transformed biological sample is prepared by treating a population of glycosaminoglycans or other glycan (e.g., glycolipid), the glycosaminoglycans or other glycan (e.g., glycolipid) being present in or isolated from a biological sample (e.g., urine, serum, plasma, PBMC or CSF) from an individual. Diagnostics, methods and compositions of matter described herein when referring to a glycosaminoglycan in general or a specific glycosaminoglycan, e.g., dermatan sulfate, chondroitin sulfate or keratan sulfate, is understood to contain disclosure for any suitable glycan (e.g., a glycolipid). In certain embodiments, the glycosaminoglycans are treated with at least one agent suitable for cleaving bonds between saccharide residues of glycosaminoglycans. In some embodiments, a process described herein comprises transforming a biological sample by treating a population of glycosaminoglycans, the glycosaminoglycans being present in or isolated from a biological sample from an individual. In certain embodiments, the glycosaminoglycans are treated with at least one agent suitable for cleaving bonds between saccharide residues of glycosaminoglycans. In specific embodiments, treating a biological sample that comprises glycosaminoglycans with at least one agent suitable for cleaving bonds between saccharide residues of glycosaminoglycans comprises treating the biological sample with one or more digesting glycosaminoglycan lyase. In some embodiments, the one or more digesting glycosaminoglycan lyase is one or more keratan sulfate digesting lyase, one or more chondroitin sulfate digesting lyase, one or more dermatan sulfate digesting

lyase, or a combination thereof. In some embodiments, the one or more digesting glycosaminoglycan lyase is one or more chondroitinase, one or more keratanase, or a combination thereof. In certain embodiments, treatment of the glycosaminoglycan with the lyase provides to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the at least one digesting glycosaminoglycan lyase is one or more keratanase (e.g., keratanase I or keratanase II). In specific embodiments, the at least one digesting glycosaminoglycan lyase is one or more chondroitinase (e.g., chondroitinase ABC, chondroitinase B, and chondroitinase AC).

[0070] In certain embodiments, the abnormal glycosaminoglycan accumulation comprises abnormal chondroitin sulfate accumulation, abnormal keratan sulfate accumulation, abnormal dermatan sulfate accumulation, or a combination thereof. In some embodiments, disorders associated with abnormal glycosaminoglycan accumulation include lysosomal storage diseases, such as mucopolysaccharidosis (MPS) (e.g., MPS IVA or MPS IVB).

[0071] In some embodiments, the process of diagnosing the identity of or the severity of a disorder associated with the accumulation of glycosaminoglycans is a disorder associated with abnormal dermatan sulfate accumulation (e.g., MPS IVA). In certain embodiments, the process of diagnosing the identity of or the severity of a disorder associated with the accumulation of glycosaminoglycans is a disorder associated with abnormal chondroitin sulfate accumulation (e.g., MPS IVA). In some embodiments, the process of diagnosing the identity of or the severity of a disorder associated with the accumulation of glycosaminoglycans is a disorder associated with abnormal keratan sulfate accumulation (e.g., MPS IVA, MPS IVB, or the like). In some embodiments, oligosaccharides provided by treating the glycosaminoglycan with a suitable glycosaminoglycan lyase are utilized in processes described herein to diagnose the identity of and/or measure the severity of a disorder associated with the abnormal accumulation of the particular glycosaminoglycan. Specific oligosaccharides provided by treating various glycosaminoglycans with glycosaminoglycan lyases are provided herein in the oligosaccharide section.

[0072] Moreover, in certain embodiments, the diagnostic methods described herein (or other method described herein) are suitable for diagnosing (or measuring the efficacy of a treatment of) a disorder in an individual involved with glycan (e.g., glycosaminoglycan) accumulation or

any disorder involved with altered glycosaminoglycan synthesis and degradation (e.g., any disorder that provides a unique glycosaminoglycan or population of glycosaminoglycans that can be detected by a process described herein). In some embodiments, such a disease includes Alzheimer's Disease, wherein glycosaminoglycans are present in plaques, and a biological sample is taken from the plaque and analyzed according to a process described herein. In other embodiments, such a disease includes cancer.

[0073] In some embodiments, specific oligosaccharides are detected and/or measured according to methods and/or processes described herein to diagnose the identity and/or severity of a specific disorder associated with glycosaminoglycan accumulation, e.g., MPS IV. In some embodiments, such oligosaccharides are described herein. In specific embodiments, a process for diagnosing the identity or severity of a disorder associated with the accumulation of glycosaminoglycans provided herein comprises detecting and/or measuring one or more oligosaccharide obtainable by digesting normal or abnormal keratan sulfate, chondroitin sulfate, dermatan sulfate, or other oligosaccharides of Formulas I-V or described in Figures 1-4. In certain embodiments, the one or more oligosaccharides detected and/or measured are free of carbon-carbon unsaturation. In some embodiments, the one or more oligosaccharides detected and/or measured are free of C4 and C5 carbon unsaturation on the saccharide residue at the non-reducing end of the oligosaccharide. In some embodiments, the oligosaccharide provided herein is monosaccharide, disaccharide or trisaccharide comprised of one, two or three saccharide residues that formed the original one, two or three saccharide residues of a glycosaminoglycan prior to treatment with the one or more glycosaminoglycan lyase. In certain instances, the amount of monosaccharide, disaccharide or trisaccharide is representative of the amount of accumulated glycosaminoglycans comprising the same monosaccharide, disaccharide or trisaccharide as residue thereof, at its non-reducing end. In other embodiments, the oligosaccharide provided herein consists of more than three saccharide residues that formed more than three original saccharide residues of a glycosaminoglycan prior to treatment with the one or more glycosaminoglycan lyase.

[0074] In certain instances, a diagnostic method described herein is useful for analyzing MPS IV, e.g., MPS IVA and MPS IVB. In some instances, the glycosaminoglycan accumulation provides a unique population of glycosaminoglycans depending on the specific MPS class. In

specific instance, the unique population of glycosaminoglycans can be identified as being correlated with a specific MPS class by detecting and/or measuring oligosaccharides in a sample taken from an individual diagnosed with or suspected of having an MPS IV disorder, the oligosaccharides being free of C4 and C5 carbon unsaturation on the saccharide residue at the non-reducing end of the oligosaccharide. In certain instances, the oligosaccharides are digested with a suitable enzyme, such as a lyase (e.g., keratan sulfate lyase, chondroitin sulfate lyase, and dermatan sulfate lyase) prior to detection/measurement and the resulting oligosaccharide (shorter in certain instances than the sample oligosaccharide, such as mono-, di- or tri-saccharides) are detected/measured. In certain instances, the degradation enzymes work by an eliminase mechanism which introduces an unsaturated bond on the newly generated non-reducing end; whereas preexisting non-reducing ends retain their full mass (e.g., these non-reducing ends are free of C4 and C5 carbon unsaturation). Thus, in certain embodiments, the digested oligosaccharides comprising non-reducing ends that are free of C4 and C5 carbon unsaturation are representative of the total number of oligosaccharides present in the original sample composition. In certain instances, the mechanism of digesting effectively tags the preexisting ends to allow for their identification by their unique mass (e.g., being 18 Daltons larger than the other oligosaccharides provided by internal oligosaccharide residues).

[0075] In certain embodiments, a process for diagnosing the identity or severity of a disorder associated with the accumulation of glycosaminoglycans provided herein comprises detecting and/or measuring one or more oligosaccharide provided herein (e.g., oligosaccharides of Formulas I-V or those shown in the Figures 1-4 described herein). In certain embodiments, the one or more oligosaccharides detected and/or measured comprise at least one point of carbon-carbon unsaturation. In some embodiments, the one or more oligosaccharides detected and/or measured comprise C4 and C5 carbon unsaturation on the saccharide residue at the non-reducing end of the oligosaccharide.

[0076] In certain embodiments, processes described herein, including diagnostic processes, include preparing a transformed biological sample by purifying a population of oligosaccharides in a biological sample that has been treated with the at least one glycosaminoglycan lyase (e.g., one or more keratanase or chondroitinase), the transformed biological sample comprising the isolated population of oligosaccharides. In some embodiments, glycosaminoglycans of the

biological sample from an individual are purified prior to treatment with the one or more glycosaminoglycan lyase.

[0077] In some embodiments, a diagnostic (including identity or severity diagnostic) process provided herein comprises comparing a detection or measurement according to the process to a control reading. In some embodiments, the comparison to a control comprises comparing the amount of the population of one or more oligosaccharide present in the transformed biological sample to an amount of a population of the one or more oligosaccharide present in a control biological sample that has been treated in a manner substantially similar to the transformed biological sample. In specific embodiments, the control biological sample was provided from an individual that does not have a disorder associated with abnormal glycosaminoglycan accumulation (e.g., MPS IV). In specific embodiments, the control biological sample was provided from an individual that has a disorder associated with abnormal glycosaminoglycan accumulation (e.g., MPS IV). In some embodiments, the control is from an individual with MPS IV. In more specific embodiments, the control is from an individual with MPS IVA. In more specific embodiments, the control is from an individual with MPS IVB.

[0078] In some embodiments, detecting the presence of or measuring the amount of a population of one or more oligosaccharide present in a transformed biological sample according to a process described herein comprises:

a. isolating a subpopulation of one or more oligosaccharides in the transformed biological sample (e.g., a transformed urine, serum, plasma, PBMC, or CSF sample); and
b. detecting the presence of and/or measuring the amount of one or more oligosaccharides present in the subpopulation.

[0079] Isolation of the subpopulation of one or more oligosaccharides in the transformed biological sample is achieved in any suitable manner, e.g., using a purification process described herein (e.g., chromatography, electrophoresis, filtration, centrifugation, etc.). Similarly, according to any process described herein, the detection of and/or measuring the presence of one or more oligosaccharide is achieved utilizing any suitable process, including those detection processes set forth herein (e.g., spectrometry, UV-Visible spectrometry, IR spectrometry, NMR spectrometry, mass spectrometry, or the like). In specific instances, prior to detecting and/or

measuring the oligosaccharide present, any process described herein further comprises tagging the reducing end of a representative portion of the one or more oligosaccharides in the transformed biological sample with any suitable detectable label (e.g., a mass label, a radio label, a fluorescent label, a chromophore label, affinity label, etc.).

[0080] In certain embodiments, the detection of the presence and/or measure of the amount of oligosaccharide is performed utilizing an analytical instrument. In specific embodiments, the analytical device comprises a spectrometer that detects and/or measures the amount of a detectable label. In certain embodiments, the detection and/or measurement of amounts of a detectable label serves as a proxy to the presence or amounts of glycosaminoglycans present. In more specific embodiments, the spectrometer includes, by way of non-limiting example, one or more of a mass spectrometer, a nuclear magnetic resonance spectrometer, a UV-Vis spectrometer, an IR spectrometer, a fluorimeter, a phosphorimeter, a radiation spectrometer, or the like. In certain embodiments, the analytical device comprises a purification device coupled to a detector or a measuring device (e.g., a HPLC system coupled to a UV-Vis spectrometer). In certain embodiments, an analytical device is a liquid chromatography mass spectrometer (LC-MS) that detects and/or measures the mass of an oligosaccharide.

[0081] In some embodiments, the presence detected and/or the measure of the population of the oligosaccharide is displayed or recorded. In some embodiments, the process comprises displaying or recording the results of the characterization. In certain embodiments, the results are displayed on a display monitor (e.g., a computer monitor, television, PDA, or the like), or print out. In some embodiments, the results are recorded on an electronic medium (e.g., a hard disk drive, magnetic storage drive, optical storage drive or the like; a disk such as a floppy disk, CD, DVD, BLU-ray or the like; a flash memory drive; removable drive or the like).

[0082] In certain embodiments, the individual is a mammal, e.g., a human. In some embodiments, the human is a newborn. In other embodiments, the human is an adult. In certain embodiments, the human is an embryo *in utero*. In some embodiments, the human has been diagnosed with a lysosomal storage disease. In some embodiments, the human is suspected of suffering from a lysosomal storage disease.

[0083] In certain embodiments, the disorder associated with abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IV, e.g., MPS IVA or MPS IVB. Thus, in

some embodiments, provided herein is a process for diagnosing the presence, identity, and/or severity of MPS IV in an individual, the process comprising the steps of:

a. generating one or more biomarkers comprising of one or more non-reducing end oligosaccharides, wherein the one or more biomarkers are oligosaccharides and are generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan enzymes, wherein after enzyme treatment, the amount of the one or more biomarkers in samples from individuals with abnormal glycosaminoglycan accumulation are different from the amount of the one or more biomarkers in samples from individuals with normal glycosaminoglycan; and

b. using an analytical instrument to detect the presence of and/or measure the amount of the one or more biomarkers produced and displaying or recording the presence of or a measure of a population of the one or more biomarkers,

wherein the presence of and/or measure the amount of the one or more biomarkers is utilized to determine the presence, identity, and/or severity of MPS IV.

[0084] In some embodiments, the process provided herein comprises generating one biomarker, and wherein the amount of the biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is different from the amount of the biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the process provided herein comprises generating one biomarker, and wherein the amount of the biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is higher than the amount of the biomarker in the samples from individuals with normal glycosaminoglycan. For example, in some embodiments, the amount of the biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, or 500-fold of the amount of the biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the amount of the biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is more than 100-fold of the amount of the biomarker in the samples from individuals with normal glycosaminoglycan.

In some embodiments, the process provided herein comprises generating one biomarker, and wherein amount of the biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is lower than the amount of the biomarker in the samples from individuals with normal glycosaminoglycan. For example, in some embodiments, the amount of the biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is about 2 %, 3 %, 4 %, 5 %, 6 %, 7 %, 8 %, 9 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, or 95% of the amount of the biomarker in the samples from individuals with normal glycosaminoglycan.

[0085] In some embodiments, the process provided herein comprises generating a first biomarker and a second biomarker, and wherein the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is different from the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

[0086] In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is more than one fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold to 100-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold to 20-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan. In some embodiments, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold to 10-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan. For example, the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-

fold, 80-fold, 90-fold, or 100-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

[0087] In a specific embodiment, the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans provided herein is MPS IVA. In another specific embodiment, the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans provided herein is MPS IVB.

[0088] In some embodiments, the at least one digesting glycosaminoglycan enzymes provided herein are lyases. In some embodiments, the at least one digesting glycosaminoglycan enzymes provided herein are non-lyases. In some embodiments, the at least one digesting glycosaminoglycan enzymes provided herein are one or more keratan sulfate digesting lyases, one or more chondroitin sulfate digesting lyases, one or more dermatan sulfate digesting lyases, or combinations thereof. In some embodiments, the at least one digesting glycosaminoglycan enzymes provided herein are one or more chondroitinase, one or more keratanase, or a combination thereof. In some embodiments, the at least one digesting glycosaminoglycan enzymes comprise one or more keratanases. In a specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise keratanase I. In another specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise keratanase II. In some embodiments, the at least one digesting glycosaminoglycan enzymes comprise one or more chondroitinase. In a specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise chondroitinase ABC. In a specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise chondroitinase AC. In a specific embodiment, the at least one digesting glycosaminoglycan enzymes comprise chondroitinase B.

[0089] Thus, in some specific embodiments, provided herein is a process for diagnosing the presence, identity, and/or severity of MPS IV (e.g., MPS IVA and MPS IVB) in an individual, the process comprising the steps of:

a. generating one or more biomarkers comprising of one or more non-reducing end oligosaccharides, wherein the one or more biomarkers are oligosaccharides and are generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan enzyme, wherein after enzyme

treatment, the amount of the one or more biomarkers in samples from individuals with abnormal glycosaminoglycan accumulation are different from the amount of the one or more biomarkers in samples from individuals with normal glycosaminoglycan; and

b. using an analytical instrument to detect the presence of and/or measure the amount of the one or more biomarkers produced and displaying or recording the presence of or a measure of a population of the one or more biomarkers,

wherein the presence of and/or measure the amount of the one or more biomarkers is utilized to determine the presence, identity, and/or severity of MPS IV; and

wherein the at least one digesting glycosaminoglycan enzyme is selected from the group consisting of keratan sulfate lyases, chondroitinase sulfate lyases, and dermatan sulfate lyases.

[0090] In some embodiments, the abnormal glycosaminoglycan accumulation provided herein comprises abnormal chondroitin sulfate accumulation, abnormal keratan sulfate accumulation, abnormal dermatan sulfate accumulation, or a combination thereof. In some embodiments, the abnormal glycosaminoglycan accumulation is abnormal keratan sulfate accumulation. In some embodiments, the abnormal glycosaminoglycan accumulation is abnormal chondroitin sulfate accumulation. In some embodiments, the abnormal glycosaminoglycan accumulation is abnormal dermatan sulfate accumulation.

[0091] In certain more specific embodiments, the abnormal glycosaminoglycan accumulation provided herein comprises abnormal chondroitin sulfate accumulation, abnormal keratan sulfate accumulation, abnormal dermatan sulfate accumulation, or a combination thereof, and the at least one digesting glycosaminoglycan enzymes provided herein are one or more keratan sulfate digesting lyases, one or more chondroitin sulfate digesting lyases, one or more dermatan sulfate digesting lyases, or combinations thereof. Thus, in some specific embodiments, provided herein is a process for diagnosing the presence, identity, and/or severity of MPS IV (e.g., MPS IVA and MPS IVB) in an individual, the process comprising the steps of:

a. generating one or more biomarkers comprising of one or more non-reducing end oligosaccharides, wherein the one or more biomarkers are oligosaccharides and are generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual,

with at least one digesting glycosaminoglycan enzyme, wherein after enzyme treatment, the amount of the one or more biomarkers in samples from individuals with abnormal glycosaminoglycan accumulation are different from the amount of the one or more biomarkers in samples from individuals with normal glycosaminoglycan; and

b. using an analytical instrument to detect the presence of and/or measure the amount of the one or more biomarkers produced and displaying or recording the presence of or a measure of a population of the one or more biomarkers,

wherein the presence of and/or measure the amount of the one or more biomarkers is utilized to determine the presence, identity, and/or severity of MPS IV;

wherein the population of glycosaminoglycans comprises a glycosaminoglycan selected from the group consisting of keratan sulfate, chondroitin sulfate, and dermatan sulfate; and

wherein the at least one digesting glycosaminoglycan enzyme is selected from the group consisting of keratan sulfate lyases, chondroitinase sulfate lyases, and dermatan sulfate lyases.

[0092] In some embodiments, the process provided herein further comprises purifying transformed biological sample using chromatography or electrophoresis. In some embodiments, the chromatography provided herein is high performance liquid chromatography (HPLC), gas chromatography (GC), column chromatography, affinity chromatography, or thin layer chromatography (TLC).

[0093] In some embodiments, the oligosaccharides provided herein are detected using mass spectrometry. In some embodiments, the process of preparing transformed biological sample further comprises tagging the reducing end of a representative portion of the one or more oligosaccharides in the transformed biological sample with a detectable label. In some embodiments, the detectable label is a mass label, a radio label, a fluorescent label, a chromophore label, or affinity label. In some embodiments, the tagged portion of the one or more oligosaccharides is detected or measured using UV-Vis spectroscopy, IR spectroscopy, mass spectrometry, or a combination thereof.

[0094] In some embodiments, the process provided herein is partially based on disease-specific biomarkers that can give a clinical readout for disease severity and response to therapy. In some embodiments, the process provided here can be used to diagnose the presence, identity, and/or

severity of abnormal keratan sulfate (KS) accumulation in an individual with MPS IV, or with MPS IVA, or with MPS IVB. In some embodiments, the enzyme used to treat the keratan sulfate is keratanase I or keratanase II. Keratanase I and keratanase II exhibit different cleavage site specificities: keratanase I cleaves after unsulfated galactose; and keratanase II cleaves after 6-sulfated GlcNAc residues within keratan sulfate chains as shown in Figure 1.

[0095] In some embodiments, the at least one digesting glycosaminoglycan enzyme is keratanase I. The structure of the non-reducing end (NRE) of KS of unaffected normal individuals is different from that of Morquio A affected individuals (individuals with MPS IVA). This structural difference is a function of the enzymatic deficiency causing the disease which results in the accumulation of KS with a 6-sulfated galactose at the NRE. The location of 6-sulfated galactose at the NRE affects the types of residues generated by keratanase I digestion. Because the enzyme only cleaves after unsulfated galactose, the oligosaccharides generated from KS of Morquio A affected individuals have a higher prevalence of 6-sulfated galactose at the NRE. This results in high levels of odd numbered oligosaccharides since the galactose residues are typically evenly spaced throughout the chain. In contrast, the oligosaccharides generated from KS of unaffected normal individuals' exhibit lower levels of odd numbered oligosaccharides relative to even numbered ones since the probability of having a 6-sulfated galactose at the NRE is much lower. Figure 2A shows a schematic illustration of the use of keratanase I to evaluate KS samples for Morquio A.

[0096] Thus, in some embodiments of the various processes provided herein, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes comprises keratan sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprise keratanase I; wherein a first biomarker and a second biomarker are generated; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IV, or is MPS IVA, or is MPS IVB.

[0097] In some embodiments, the first biomarker is odd numbered oligosaccharide, and the second biomarker is even numbered oligosaccharide.

[0098] As used herein, the term "odd numbered oligosaccharide" means a monosaccharide or a saccharide polymer containing odd numbered monosaccharide units. Exemplary odd numbered oligosaccharides include, but not limited to, a saccharide polymer containing 3, 5, 7, 9, 11, or 13

monosaccharide units. For example, in some embodiments, the odd numbered oligosaccharide is a trisaccharide. In some embodiments, the odd numbered oligosaccharide has a non-reducing end of Gal6S. In some embodiments, the odd numbered oligosaccharide having a non-reducing end of Gal6S has a formula of Gal6S-(X)n-Gal (Formula I), wherein X is a monosaccharide residue and n=an odd number such as 1, 3, 5, 7, and 9. Exemplary oligosaccharides having a formula of Gal6S-(X)n-Gal include, but not limited to, Gal6S- GlcNAc6S-Gal, Gal6S- GlcNAc6S-Gal6S-GlcNAc6S-Gal, and Gal6S- GlcNAc6S-Gal6S- GlcNAc6S-Gal6S- GlcNAc6S-Gal. As used herein, the term "even numbered oligosaccharide" means a saccharide polymer containing even numbered monosaccharide units. Exemplary even numbered oligosaccharides include, but not limited to, a saccharide polymer containing 2, 4, 6, 8, 10, or 12 monosaccharide units. For example, in some embodiments, the even numbered oligosaccharide is a tetrasaccharide.

[0099] In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is higher than the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 100-fold of the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 20-fold of the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. For example, in some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold of the ratio of the amount of the odd numbered oligosaccharides to

the amount of the even numbered oligosaccharides in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB.

[00100] In some embodiments, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is more than 1:5. Exemplary ratios of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB include, but not limited to, about 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, and 10:1.

[00101] In a specific embodiment, the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 1:2; and the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB is 1:5.

[00102] In some embodiments of the various processes described above, the individual has MPS IVA.

[00103] In other embodiments, the at least one digesting glycosaminoglycan enzyme is keratanase II. Keratanase II cleaves after 6-sulfated GlcNAc residues within the KS chains resulting in the release of disaccharides and small oligosaccharides (typically tetrasaccharides) with a 6-sulfated GlcNAc at the reducing end. Keratanase II digestion usually results in oligosaccharides with both sulfated and unsulfated galactose at the NRE. Morquio A patients exhibit KS with a higher proportion of 6-sulfated galactose at the NRE relative to normal individuals. Thus, the relative proportion of oligosaccharides with a 6-sulfated terminus increases in the affected individuals with MPS IVA as illustrated in Figure 3.

[00104] Thus, in some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises keratan sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprises keratanase II; wherein a first biomarker and a second biomarker are generated; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IV, or is MPS IVA, or is MPS IVB.

[00105] In some embodiments, the first biomarker is an oligosaccharide with sulfated galactose at the non-reducing end; and the second biomarker is an oligosaccharide with unsulfated galactose at the non-reducing end. In some embodiments, the first biomarker and the second biomarker are tetrasaccharides. Exemplary oligosaccharides with sulfated galactose at the non-reducing end include, but not limited to, Gal6S- GlcNAc6S-Gal- GlcNAc6S. The second and the forth monosaccharide unit in this tetrasaccharide can also be sulfated at other positions or unsulfated, and thus each of them can independently be, *e.g.*, GlcNAc2S, GlcNAc3S, GlcNAc4S, GlcNS, or GlcNAc. Similarly, the third monosaccharide Gal in this tetrasaccharide can be sulfated as, *e.g.*, Gal3S, Gal4S, or Gal6S. Exemplary oligosaccharides with unsulfated galactose at the non-reducing end include, but not limited to, Gal-GlcNAc6S-Gal6S-GlcNA6S. The second and the forth monosaccharide unit in this tetrasaccharide can also be sulfated at other positions or unsulfated, and thus each of them can independently be, *e.g.*, GlcNAc2S, GlcNAc3S, GlcNAc4S, GlcNS, or GlcNAc. Similarly, the third monosaccharide Gal in this tetrasaccharide can be sulfated in other positions or unsulfated, and thus can be, *e.g.*, Gal3S, Gal4S, or Gal.

[00106] In some embodiments, the oligosaccharide with sulfated galactose at the non-reducing end has a formula of Gal6S-(X)n-GlcNAc6S (Formula II), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. Exemplary oligosaccharides having a formula of Gal6S-(X)n-GlcNAc6S include, but not limited to, Gal6S-GlcNAc6S-Gal- GlcNAc6S, Gal6S- GlcNAc-Gal- GlcNAc6S, Gal6S- GlcNAc2S-Gal-GlcNAc6S, Gal6S- GlcNAc3S-Gal- GlcNAc6S, Gal6S- GlcNAc4S-Gal- GlcNAc6S, Gal6S-GlcNS-Gal- GlcNAc6S, Gal6S- GlcNAc6S-Gal3S- GlcNAc6S, Gal6S- GlcNAc6S-Gal4S-GlcNAc6S, and Gal6S- GlcNAc6S-Gal6S- GlcNAc6S.

[00107] In some embodiments, the oligosaccharide with unsulfated galactose at the non-reducing end has a formula of Gal-(X)n-GlcNAc6S (Formula III), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. Exemplary oligosaccharides having a formula of Gal-(X)n-GlcNAc6S include, but not limited to, Gal-GlcNAc6S-Gal- GlcNAc6S, Gal- GlcNAc-Gal- GlcNAc6S, Gal- GlcNAc2S-Gal- GlcNAc6S, Gal- GlcNAc3S-Gal- GlcNAc6S, Gal- GlcNAc4S-Gal- GlcNAc6S, Gal- GlcNS-Gal-

GlcNAc6S, Gal- GlcNAc6S-Gal3S- GlcNAc6S, Gal- GlcNAc6S-Gal4S- GlcNAc6S, and Gal-GlcNAc6S-Gal6S- GlcNAc6S.

[00108] In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is higher than the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 1000-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 500-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 200-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is

2-fold to 100-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 20-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold to 10-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB. For example, in some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 150-fold, 200-fold, 500-fold, or 1000-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB.

[00109] In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is higher than 1.5:1. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals

with MPS IV, or with MPS IVA, or with MPS IVB is 1.5:1 to 100:1. Exemplary ratios of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB include, but not limited to, about 1.5:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, and 100:1.

[00110] In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB is 1.5:1 or lower. In some embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB is 1.5:1 to 0.5:1. Exemplary ratios of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB include, but not limited to, about 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1.0:1, 0.9:1, 0.8:1, 0.7:1, 0.6:1, and 0.5:1.

[00111] In a specific embodiments, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IV, or with MPS IVA, or with MPS IVB is 10:1; and the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IV, or without MPS IVA, or without MPS IVB is 1:1.

[00112] In some embodiments of the various processes described above, the individual has MPS IVA.

[00113] In yet other embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises chondroitin sulfate (CS) or dermatan sulfate (DS). As illustrated in Figure 4A, lyase digestion can liberate the disease specific NRE. In some embodiments, the MPS IVA specific NRE is 6-O sulfated

GalNAc. As shown in Figure 4B, chondroitinase ABC digestion of CS/DS samples results in an increased level of 6-sulfated GalNAc as well as an increase in ratio of 6-sulfated GalNAc relative to 4-sulfated GalNAc4S in MPS IVA samples compared to that in unaffected samples.

[00114] Thus, in some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises chondroitin sulfate or dermatan sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprise chondroitinase ABC; wherein a first biomarker and a second biomarker are generated; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IVA. In some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises chondroitin sulfate. In some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes provided herein comprises dermatan sulfate.

[00115] In some embodiments, the first biomarker is an oligosaccharide with 6-sulfated GalNAc at the non-reducing end; and the second biomarker is an oligosaccharide with 4-sulfated GalNAc at the non-reducing end. In some embodiments, the oligosaccharide with 6-sulfated GalNAc at the non-reducing end has a formula of GalNAc6S-(X)n (Formula IV), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. In some embodiments, the oligosaccharide having a formula of GalNAc6S-(X)n (Formula IV) is GalNAc6S. In other embodiments, the oligosaccharide with 4-sulfated GalNAc has a formula of GalNAc4S-(X)n (Formula V), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. In some embodiments, the oligosaccharide having a formula of GalNAc4S-(X)n (Formula V) is GalNAc4S.

[00116] In a specific embodiment, the first biomarker is 6-sulfated GalNAc, and the second biomarker is 4-sulfated GalNAc.

[00117] In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is higher than the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to

the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA.

[00118] In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 100-fold of the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 20-fold of the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA. In some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 10-fold of the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA. For example, in some embodiments, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold of the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA.

[00119] In a specific embodiment, the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is more than 10:1; and the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing

end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA is less than 1:1.

[00120] As discussed above and shown in Figure 4, chondroitinase ABC digestion of CS/DS samples results in an increased level of 6-sulfated GalNAc compared to that in unaffected samples. This result has been further confirmed in a study comparing normal adult individuals versus adult MPS IVA patients, in which the level of 6-sulfated GalNAc in MPS IVA patients is more than 100-fold of that of normal individuals (see Figure 5). Accordingly, the level of 6-sulfated GalNAc post enzyme digestion with chondroitinase can be used to diagnose a patient with MPS IVA.

[00121] Thus, in some embodiments, the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzymes comprises chondroitin sulfate or dermatan sulfate; wherein the at least one digesting glycosaminoglycan enzymes comprises chondroitinase ABC; wherein a biomarker is generated, the biomarker being 6-sulfated GalNAc; and wherein the disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans is MPS IVA.

[00122] In some embodiments, the method provided herein further comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is higher than a reference level of 6-sulfated GalNAc.

[00123] In some embodiments, the reference level of 6-sulfated GalNAc is determined by using a control sample obtained from a normal individual without MPS IVA. In other embodiments, the reference level of 6-sulfated GalNAc is determined by using a control sample obtained from the same individual before digestion with chondroitinase ABC.

[00124] In a specific embodiment, provided herein is a process for diagnosing the presence, identity, and/or severity of MPS IVA in an individual, the process comprising the steps of:

a. obtaining a biological sample from an individual;

b. treating a population of chondroitin sulfate or dermatan sulfate, in or isolated from the biological sample, with chondroitinase ABC;

c. using an analytical instrument to measure the amount of 6-sulfated GalNAc produced and displaying or recording a measure of 6-sulfated GalNAc;

d. comparing the level of 6-sulfated GalNAc in the biological sample with a reference level of 6-sulfated GalNAc; and

e. diagnosing the individual as having MPS IVA if the level of 6-sulfated GalNAc in the biological sample from the individual is higher than the reference level of 6-sulfated GalNAc.

[00125] In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is 2-fold to 2000-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is 2-fold to 1000-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 110-fold, 120-fold, 130-fold, 140-fold, or 150-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 20-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 50-fold of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 100-fold of the reference level of 6-sulfated GalNAc.

[00126] In some embodiments, the individual is an adult. In other embodiments, the individual is a new-born.

[00127] In some embodiments, provided herein is a process of treating an abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., MPS IV, comprising administering a treatment agent to the individual determined to have abnormal glycosaminoglycan accumulation, or a disorder thereof, e.g., MPS IV, using the various processes provided herein. As such, in some embodiments, provided herein is a process of

treating abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, e.g., MPS IV, the process comprising the steps of:

a) generating one or more biomarkers comprising of one or more non-reducing end oligosaccharides, wherein the one or more biomarkers are oligosaccharides and are generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan enzymes, wherein after enzyme treatment, the amount of the one or more biomarkers in samples from individuals with abnormal glycosaminoglycan accumulation are different from the amount of the one or more biomarkers in samples from individuals with normal glycosaminoglycan, and

b) using an analytical instrument to detect the presence of and/or measure the amount of the one or more biomarkers produced and displaying or recording the presence of or a measure of a population of the one or more biomarkers;

c) using the presence of and/or measure the amount of the one or more biomarkers to determine the presence, identity, and/or severity of abnormal glycosaminoglycan accumulation; and

d) administering a treatment agent to the individual determined to have abnormal glycosaminoglycan accumulation, or a disorder thereof, e.g., MPS IV.

[00128] In some specific embodiments, the process provided herein further comprises administering a treatment agent to the individual diagnosed as having MPS IVA using the processes provided herein. In some embodiments, the treatment agent is an enzyme replacement therapy (ERT). In some embodiments, in an ERT, an enzyme in an individual, e.g., the enzymes that are deficient or absent, is replaced. In some embodiments, the ERT is administered by intravenous infusion, e.g., at dosages based on patient body weight. In some embodiments, the ERT is achieved through gene therapy, e.g., by delivering a nucleic acid encoding the desired enzyme to a target region of the individual. In other embodiments, the ERT comprises rescuing mutated enzymes with pharmacological chaperones. In other embodiments, the ERT comprises stimulating transcription or translation of an enzyme, e.g., an absent enzyme. In yet other

embodiments, the ERT comprises activating stop codon readthrough. In more specific embodiments, the treatment agent is elosulfase alfa (VIMIZIM®).

[00129] In other embodiments, the treatment agent is bone marrow transplantation (BMT). In other embodiments, the treatment agent is umbilical cord blood transplantation (UCBT). Other treatment agents known to those skilled in the art for treating MPS IVA are also included in the present disclosure.

[00130] In some embodiments, the treatment agent, e.g., elosulfase alfa, is administered from about 0.001 to about 100 mg/kg/day (mg per kg body weight per day), from about 0.01 to about 50 mg/kg/day, from about 0.01 to about 25 mg/kg/day, from about 0.01 to about 10 mg/kg/day, from about 0.01 to about 9 mg/kg/day, 0.01 to about 8 mg/kg/day, from about 0.01 to about 7 mg/kg/day, from about 0.01 to about 6 mg/kg/day, from about 0.01 to about 5 mg/kg/day, from about 0.01 to about 4 mg/kg/day, from about 0.01 to about 3 mg/kg/day, from about 0.5 to about 3 mg/kg/day, or from about 1.0 to about 2.5 mg/kg/day.

[00131] In some embodiments, the treatment agent, e.g., elosulfase alfa, is administered once a day, twice a week, once a week, once every two weeks, once every three weeks, or once every four weeks.

[00132] Depending on the disorder to be treated and the subject's condition, the treatment agent may be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, CIV, intracistemal injection or infusion, subcutaneous injection, or implant), inhalation, nasal, vaginal, rectal, sublingual, or topical (e.g., transdermal or local) routes of administration. In some specific embodiments, the treatment agent, e.g., elosulfase alfa, is administered as an intravenous infusion.

[00133] In some embodiments of the various processes provided herein, the oligosaccharide provided herein is selected from a group consisting of monosaccharide, disaccharide, and trisaccharide. In a specific embodiment, the oligosaccharide provided herein is a monosaccharide. In other embodiments, the oligosaccharide provided herein comprises more than three monosaccharide units.

[00134] In some embodiments, patient samples can be acquired and subjected to digestion with pronase solution for 24 h. The digests are then diluted with water to reduce salt concentration and the glycosaminoglycan fraction is purified by anion exchange chromatography

(DEAE-sephacyl) and eluted with high salt. The eluted glycosaminoglycan fraction is then desalted by gel permeation chromatography (PD-10) and dried down. Then glycosaminoglycan can be reconstituted in water and suitable aliquots can be subjected to depolymerization followed by derivatization of the reducing end with appropriate mass (such as aniline) or chromophoric (such as AMAC or 2AB) tags by reductive amination with sodium cyanoborohydride under aprotic conditions. Analysis and identification of disease specific biomarkers can then be carried out. In some embodiments, analysis and identification of disease specific biomarkers can be carried out by LC/MS. In the LC/MS format, LC separation is carried out by reverse phase ion pairing HPLC or UPLC, and MS detection is carried out in a negative ion mode. Elution can be carried out with increasing methanol. Dibutyl amine can be used as the ion pairing reagent allowing sulfated oligosaccharides to interact with the aliphatic solid phase of C18 columns.

[00135] In another aspect, disclosed herein are biomarkers for diagnosing the presence, identity, and/or severity of abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof. In some embodiments, the biomarker is an oligosaccharide. The oligosaccharides provided herein include, but not limited to, monosaccharide and saccharide polymers containing 2, 3, 4, 5, 6, 7, 8, or 9 monosaccharide units. In some embodiments, the oligosaccharide is a monosaccharide. In some embodiments, the oligosaccharide is a disaccharide. In other embodiments, the oligosaccharide is a trisaccharide. In other embodiments, the oligosaccharide is a tetrasaccharide. In yet other embodiments, the oligosaccharide is a pentasaccharide. In yet other embodiments, the oligosaccharide is a hexasaccharide.

[00136] In some embodiments, the biomarkers provided herein can be used to diagnose the presence, identity, and/or severity of an MPS IV disorder, e.g., MPS IVA or MPS IVB.

[00137] In some embodiments, the biomarkers are generated by digesting one or more glycosaminoglycans. In some embodiments, the biomarkers are generated by digesting chondroitin sulfate. In other embodiments, the biomarkers are generated by digesting keratan sulfate. In some embodiments, the biomarkers are generated by digesting dermatan sulfate.

Analytical Samples

[00138] Provided in certain embodiments herein are compositions comprising any one or more oligosaccharides provided herein. In some embodiments, the composition provided herein

is an analytical sample, suitable analysis in any analytical device, e.g., one provided herein (such as, by way of non-limiting example, high performance liquid chromatography, mass spectrometry, gas chromatography, or the like).

[00139] In certain embodiments, a composition provided herein comprises at least one monosaccharide, disaccharide or trisaccharide from a transformed biological sample from an individual with a disorder associated with abnormal glycosaminoglycan accumulation. In specific embodiments, the transformed biological sample was prepared by treating a biological sample comprising glycosaminoglycans with one or more digesting glycosaminoglycan lyase.

[00140] In some embodiments, an analytical sample provided herein comprises one or more oligosaccharide of the oligosaccharides of Formulas I-V or shown in Figures 1-4 or any oligosaccharides obtainable by digesting normal or abnormal keratan sulfate, chondroitin sulfate, or dermatan sulfate. In certain embodiments, an analytical sample provided herein comprises one or more oligosaccharide of the oligosaccharides of Formulas I-V or shown in Figures 1-4 or any oligosaccharides obtainable by digesting normal or abnormal keratan sulfate, chondroitin sulfate, or dermatan sulfate, wherein the one or more oligosaccharides further comprise a detectable label attached (e.g., covalently and/or non-covalently) to the reducing end of the one or more oligosaccharide.

[00141] In some embodiments, provided herein is a composition comprising isolated glycans, wherein the glycans were isolated from a biological sample, and one or more glycan degradation enzyme. In certain embodiments, the composition further comprises one or more biomarker generated according to any method described herein (e.g., wherein the biomarker is a non-reducing saturated oligosaccharide). In certain embodiments, provided herein is an oligosaccharide described herein (e.g., a labeled or non-labeled non-reducing saturated oligosaccharide) and an analytical instrument or chromatographic resin.

Oligosaccharides

[00142] In certain embodiments, methods and processes described herein are utilized to detect and/or measure one or more biomarker. In specific embodiments, such biomarkers comprise one or more oligosaccharides (e.g., disaccharide(s) and/or trisaccharide(s)). In certain embodiments, the one or more oligosaccharides comprise any one or more of the oligosaccharides described herein.

[00143] As used herein, IdoA and  are iduronic acid (e.g., α-L-iduronic acid) saccharide residues. As used herein, GlcA and  are glucuronic acid (e.g., β-L-glucuronic acid) saccharide residues. As used herein,  is either an iduronic acid (e.g., α-L-iduronic acid) saccharide residue or a glucuronic acid (e.g., β-L-glucuronic acid) saccharide residue. As used herein, GlcN and  are glucosamine (e.g., 2-deoxy-2-amino-β-D-glucopyranosyl) saccharide residues. As used herein, $GlcN(Ac)_1$ and  are a glucosamine (e.g., 2-deoxy-2-amino-β-D-glucopyranosyl) saccharide residue wherein the 2-amino group is acetylated. As used herein, Gal and  is a galactose saccharide residue. As used herein GalNAc and  represents an N-acetylgalactosamine residue. As used herein  and  both represent N-sulfated (*i.e.,* N-substituted with SO_3R as described herein) glucosamine (e.g., 2-deoxy-2-amino-β-D-glucopyranosyl) saccharide residue. In various specific instances, iduronic acid, glucuronic acid, glucosamine, and/or galactose saccharide residues are saturated at 4 and 5 carbons of the non-reducing end saccharide residue, or are free of carbon-carbon unsaturation. In other instances, any one or more of the saccharide residues is unsaturated, e.g., at the 4 and 5 carbon positions of the saccharide residue at the non-reducing end of an oligosaccharide provided herein. The symbolic nomenclature used herein follows the "Symbol and Text Nomenclature for Representation of Glycan Structure" as promulgated by the Nomenclature Committee for the Consortium for Functional Glycomics, as amended on October 2007. Recitation of an NS (e.g., above or below any of the aforementioned structures) indicates that the amino group thereof is substituted with (SO_3R). If the NS is associated with $GlcN(Ac)_m$ or above or below  , the residue is $GlcN(SO_3R)$, wherein the amino group bears the (SO_3R). Recitation of a 2S (e.g., above or below any of the aforementioned structures) indicates that the hydroxyl group at the two carbon position of the indicated saccharide residue is substituted with (SO_3R). Recitation of a 3S (e.g., above or below any of the aforementioned structures) indicates that the hydroxyl

group at the three carbon position of the indicated saccharide residue is substituted with (SO_3R). Recitation of a 4S (e.g., above or below any of the aforementioned structures) indicates that the hydroxyl group at the four carbon position of the indicated saccharide residue is substituted with (SO_3R). Recitation of a 6S (e.g., above or below any of the aforementioned structures) indicates that the hydroxyl group at the six carbon position of the indicated saccharide residue is substituted with (SO_3R).

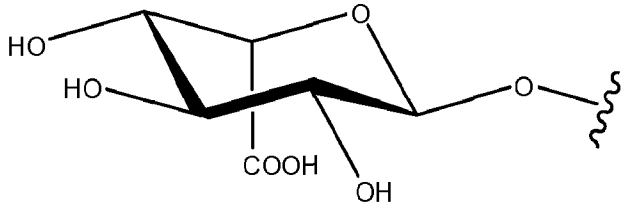
[00144] In some embodiments, the oligosaccharide is generated by digesting keratan sulfate with keratanase I. In some embodiments, methods and processes described herein are utilized to detect and/or measure an oligosaccharide having a non-reducing end of Gal6S. In some embodiments, the oligosaccharide having a non-reducing end of Gal6S is an odd numbered oligosaccharide. In certain embodiments, the oligosaccharide having a non-reducing end of Gal6S has a formula of Gal6S-(X)n-Gal (Formula I), wherein X is a monosaccharide residue and n=an odd number such as 1, 3, 5, 7, and 9. Exemplary oligosaccharides having a formula of Gal6S-(X)n-Gal include, but not limited to, Gal6S- GlcNAc6S-Gal, Gal6S- GlcNAc6S-Gal6S-GlcNAc6S-Gal, and Gal6S- GlcNAc6S-Gal6S- GlcNAc6S-Gal6S- GlcNAc6S-Gal.

[00145] In other embodiments, the oligosaccharide is generated by digesting keratan sulfate with keratanase II. In some embodiments, methods and processes described herein are utilized to detect and/or measure an oligosaccharide having a formula of Gal6S-(X)n-GlcNAc6S (Formula II), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. Exemplary oligosaccharides having a formula of Gal6S-(X)n-GlcNAc6S include, but not limited to, Gal6S- GlcNAc6S-Gal- GlcNAc6S, Gal6S- GlcNAc-Gal- GlcNAc6S, Gal6S-GlcNAc2S-Gal- GlcNAc6S, Gal6S- GlcNAc3S-Gal- GlcNAc6S, Gal6S- GlcNAc4S-Gal-GlcNAc6S, Gal6S- GlcNS-Gal- GlcNAc6S, Gal6S- GlcNAc6S-Gal3S- GlcNAc6S, Gal6S-GlcNAc6S-Gal4S- GlcNAc6S, and Gal6S- GlcNAc6S-Gal6S- GlcNAc6S. In certain embodiments, methods and processes described herein are utilized to detect and/or measure an oligosaccharide having a formula of Gal-(X)n-GlcNAc6S (Formula III), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. Exemplary oligosaccharides having a formula of Gal-(X)n-GlcNAc6S include, but not limited to, Gal-GlcNAc6S-Gal- GlcNAc6S, Gal- GlcNAc-Gal- GlcNAc6S, Gal- GlcNAc2S-Gal- GlcNAc6S, Gal- GlcNAc3S-Gal- GlcNAc6S, Gal- GlcNAc4S-Gal- GlcNAc6S, Gal- GlcNS-Gal-

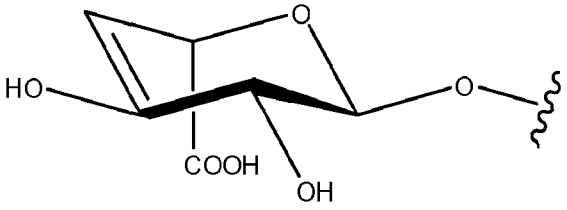
GlcNAc6S, Gal- GlcNAc6S-Gal3S- GlcNAc6S, Gal- GlcNAc6S-Gal4S- GlcNAc6S, and Gal-GlcNAc6S-Gal6S- GlcNAc6S.

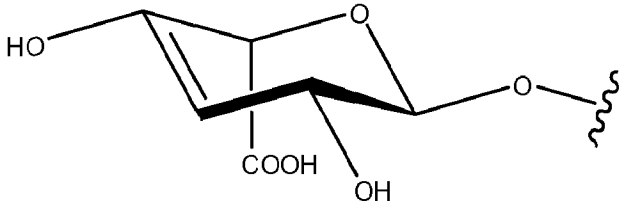
[00146] In yet other embodiments, the oligosaccharide is generated by digesting chondroitin sulfate or dermatan sulfate with chondroitinase ABC. In some embodiments, methods and processes described herein are utilized to detect and/or measure an oligosaccharide having a formula of GalNAc6S-(X)n (Formula IV), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. In some embodiments, the oligosaccharide having a formula of GalNAc6S-(X)n (Formula IV) is GalNAc6S. In other embodiments, methods and processes described herein are utilized to detect and/or measure an oligosaccharide having a formula of GalNAc4S-(X)n (Formula V), wherein X is a monosaccharide residue and n=an integer such as 0, 1, 2, 3, 4, 5, 6, 7, and 8. In some embodiments, the oligosaccharide having a formula of GalNAc4S-(X)n (Formula V) is GalNAc4S.

[00147] In certain embodiments, provided herein is a compound of any of Formulas I-V. In some embodiments, provided herein is a compound shown in Figures 1-4. In certain embodiments, the compound of any of Formulas I to V or shown in Figures 1-4 is an isolated and/or purified compound. In some embodiments, the isolated and/or purified compound is substantially free of oligosaccharides comprising a saccharide residue at the non-reducing end of the oligosaccharide that is unsaturated at the C4 and C5 positions. As an illustrative example of non-reducing end saccharide residues that are saturated and unsaturated at the C4 and C5 positions, an L-iduronic acid (IdoA) residue that is saturated at the C4 and C5 positions has a structure as follows:



whereas an L-iduronic acid (IdoA) residue at the non-reducing end of the oligosaccharide that is unsaturated at the C4 and C5 positions may have a structure as follows:



,


,

or the like. Oligosaccharides having non-reducing end saccharide residues that are saturated at the C4 and C5 position are referred to herein as "C4-C5 non-reducing end saturated oligosaccharides".

[00148] In certain embodiments, unsaturated saccharide residues include the unsaturated residue of IdoA, which is denoted by  ; and the unsaturated residue of GlcA which is denoted by  .

[00149] In some embodiments, the isolated and/or purified compound described herein comprises at least at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% oligosaccharide (by weight). In further or alternative embodiments, the isolated and/or purified compound comprises 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% NRE-saturated oligosaccharide (by weight).

[00150] In some embodiments, provided herein is a composition comprising one or more isolated oligosaccharide provided herein. In certain embodiments, the oligosaccharide present in the composition is less than 90% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 80% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 70% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 60% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 50% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 40% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 30% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in

the composition is less than 25% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 20% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 15% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 10% by weight non-reducing end unsaturated oligosaccharide. In certain embodiments, the oligosaccharide present in the composition is less than 5% by weight non-reducing end unsaturated oligosaccharide. In some embodiments, the composition further comprises chromatographic resin or electrophoretic resin. In some embodiments, the resin is a high performance liquid chromatographic resin, or the like.

[00151] The compounds of any of Formulas I-V or shown in Figures 1-4 are obtained in any suitable manner, e.g., by generation and purification from natural sources, chemical synthesis, or any other suitable method. These structures do not naturally exist in an isolated and/or purified form.

[00152] The described mono-, di- and tri-saccharides are optionally generated and purified from natural sources. In certain embodiments, starting with glycosaminoglycans from natural sources, these mono-, di- and tri-saccharides are generated by treating the glycan with glycosaminoglycan lyases (for example, keratan sulfate digesting lyases, chondroitin sulfate digesting lyases, and dermatan sulfate digesting lyases) and purifying the mono-, di- and tri-saccharides liberated from the pre-existing non-reducing ends. In specific instances, the mono-, di- and tri-saccharides liberated from the pre-existing non-reducing end are unique because they do not contain a c4-5 double bond due to the action of the lyase.

[00153] In certain embodiments, the yield of the desired mono-, di- and tri-saccharides are increased by using glycosaminoglycans that have been treated with glycan degradative enzymes (for example, heparanase or lysosomal exo-enzymes such as the 2-sulfatase, N-sulfatase, etc) to generate smaller fragments or fragments with a greater number of desirable non-reducing ends. In further or alternative embodiments, the yield is increased by starting with glycosaminoglycans that have been isolated from an organism or cell that has a defect in glycosaminoglycan degradation such that the desirable non-reducing ends are enriched (for example, glycosaminoglycans from iduronidase deficient systems, would be enriched in

glycosaminoglycans that terminal on the non-reducing end with iduronate residues). These unique non-reducing end saccharides can be isolated by HPLC. Additional or alternative methods of obtaining such compounds include, e.g., chemical transformation of the unsaturated saccharides into the saturated saccharides. The described mono-, di- and tri-saccharides can be synthesized by chemical or chemoenzymatic methods. Chemical methods for the synthesis of these saturated mono-, di- and tri-saccharides are optionally converted from the methods described by Prabhu, Venot, and Boons (Organic Letters 2003 Vol. 5, No. 26 4975-4978), which is incorporated herein for such synthesis.

[00154] In further embodiments, provided herein is any compound provided herein, wherein the compound is tagged with any label as described herein.

[00155] In some embodiments, an isolated or otherwise generated oligosaccharide described herein has a molecular weight of less than 2000 g/mol, less than 1500 g/mol, less than 1000 g/mol, less than 500 g/mol, less than 400 g/mol, less than 300 g/mol, less than 260 g/mol, less than 200 g/mol, less than 100 g/mol, or the like (e.g., prior to tagging with any detectable label that may be included in a process described herein).

Detection/Measurement Methods

[00156] Oligosaccharides (including e.g., oligosaccharides tagged with detectable labels) described herein are detected and/or measured in processes described herein in any suitable manner. In certain embodiments, detectable labels are detected and/or quantified according to any process described herein using any technique, particularly any technique suitable for the detectable label utilized. In some embodiments, suitable detection techniques include, by way of non-limiting example, one or more of a mass spectrometer, a nuclear magnetic resonance spectrometer, a UV-Vis spectrometer, an IR spectrometer, a fluorimeter, a phosphorimeter, a radiation spectrometer (e.g., a scintillation counter), a thin layer chromatographic technique, or the like. In certain embodiments, in any process described herein, oligosaccharides are optionally directly detected using a suitable technique, such as quantitative nuclear magnetic resonance. Quantitative nuclear magnetic resonance is also optionally utilized to quantify and/or detect the presence of a detectable label. In certain embodiments, one or more oligosaccharides are optionally detected using a suitable liquid chromatography mass spectrometer (LC-MS).

[00157] In some embodiments, oligosaccharides are tagged with an antibody or probe, and are quantified using any suitable method (e.g., dot blot techniques, immune detection techniques (e.g., ELISA), or the like).

[00158] Various analytical methods useful for the processes described herein include, by way of non-limiting example, mass spectrometry, chromatography, HPLC, UPLC, TLC, GC, HPAEC-PAD, electrophoresis – capillary or gel, or the like. In certain embodiments, wherein a chromatographic technique is utilized, any suitable solvent system is optionally employed. In certain embodiments, a column (e.g., Cosmogel DEAE, Tsk Gel DEAE, Cosmogel QA, Cosmogel CM, Cosmogel SP, 130A BEH particle Phenyl (1.7, 2.5, 3.5, 5, or 10 uM particle size), 130A BEH particle C18 (1.7, 2.5, 3.5, 5, or 10 uM particle size), HSS particle C18 (1.8, 3.5, or 5 uM particle size), 300A BEH particle C18 (1.7, 3.5, 5, 10 uM particle size), or the like with suitable length and internal diameter) is optionally loaded with an equilibrating solvent (e.g., a buffer or salt solution, such as a potassium acetate solution, sodium chloride solution, sodium acetate solution, ammonium acetate solution, or the like), e.g., with a pH of about 6, 7, or 8. In some embodiments, the buffer or salt solution has a concentration of about 10 mM, 20 mM, 30 mM, 50 mM, 100 mM, 500 mM, 1 M, 2 M, or the like. Any suitable flow rate is used, e.g., 0.5 mL/min, 1 mL, min, 1.5 mL/min, 2 mL/min, or the like. Following equilibration, a linear gradient is optionally utilized. In some embodiments, the linear gradient is run over 1-20 min, 1-10 min, 10-20 min, 1-5 min, 5-10 min, or the like. In certain embodiments, the gradient is a buffer or salt solution, e.g., as described above (e.g., from 0 M to 0.5 M, from 0 M to 3 M, from 0.5 M to 2 M, from 0 M to 2 M, from 1 M to 2 M, from 0 M to 3 M, from 2 M to 0 M, from 3 M to 0 M, or the like). Once the gradient has reached a final concentration, the eluent is optionally held at the final concentration for a suitable period of time (e.g., 1-20 min, 5-10 min, 10-15 min, 1-5 min, 1-10 min, 15-20 min, or the like). After the optional holding of the final concentration, the eluent may be switched to a second solvent or solvent system (e.g., an alcohol, such as methanol, ethanol, or isopropanol, acetonitrile, water, or the like). The switch to the second solvent system may be over a period of time, e.g., 15 seconds, 30 seconds, 45 seconds, 60 seconds, 2 min, 3 min, or the like. The second solvent system is optionally held for a period of time, such as 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, or the like. Following the second solvent system cycle, the column is optionally restored to initial solvent conditions.

[00159] In certain embodiments, detection or measurement of an analytical device provides for diagnosis of a disease, diagnosis of severity of a disease, of efficacy of a therapy, or analysis based on other processes described herein. For example, in some embodiments, absence of a peak or signal (e.g., a peak or signal indicative of the presence of a particular oligosaccharide) indicates that an individual is in a non-diseased state, in remission for a disease state, or undergoing effective therapy for a disease, depending on the circumstances of the diagnosis. In certain embodiments, the presence and/or area of a peak or signal (including, e.g., the presence of a certain signal or peak pattern or fingerprint) is utilized to determine the severity of a disease. In some embodiments, the presence and/or area of a peak or signal is utilized to determine disease, disease severity, disease carrier status or the like, based on a certain threshold value for the disease, disease severity, disease carrier status. Such thresholds are optionally determined in any suitable manner, e.g., by analyzing control samples, such control samples coming from non-diseased individuals, diseased individuals, or the like.

[00160] In certain embodiments, a control biological sample utilized in any process described herein was provided from an individual that does not suffer from a disorder being diagnosed. In other embodiments, a control biological sample is taken from an individual suffering from a disorder being diagnosed. In certain embodiments, the result obtained from the control biological sample is stored in a database. In such cases a test sample is optionally compared to a plurality of control data in a database. Moreover in certain embodiments, any diagnostic process described herein is optionally utilized alone or in combination with other diagnostic techniques. Other diagnostic techniques include, by way of non-limiting example, symptom analysis, biopsies, detection of accumulation of other compounds in biological samples, or the like. In some embodiments, control biological samples are optionally taken from the same individual at substantially the same time, simply from a different location (e.g., one inflamed/arthritic synovial joint fluid vs the contralateral non-arthritic synovial joint). In other embodiments, control biological samples are optionally taken from the same individual at different points in time (e.g., before therapy and after therapy if the method being utilized is a method of monitoring a treatment therapy).

Detectable Labels

[00161] In the various embodiments of any process or method described herein, any suitable detectable label is optionally utilized. In some embodiments, detectable labels useful in the processes or methods described herein include, by way of non-limiting example, mass labels, antibodies, affinity labels, radiolabels, chromophores, fluorescent labels, or the like.

[00162] Fluorescent labels suitable for use in various embodiments herein include, by way of non-limiting example, 2-aminopyridine (2-AP), 2-aminobenzoic acid (2-AA), 2-aminobenzamide (2-AB), 2-aminoacridone (AMAC), p-aminobenzoic acid ethyl ester (ABEE), p-aminobenzonitrile (ABN), 2-amino-6-cyanoethylpyridine (ACP), 7-amino-4-methylcoumarine (AMC), 8-aminonaphthalene-1,3,6-trisulfate (ANTS), 7-aminonaphthalene-1,3-disulfide (ANDS), and 8-aminopyrene-1,3,6-trisulfate (APTS), or the like. The fluorescent labels can be attached by reductive amination with the fluorescent label and sodium cyanoborohydride or the like.

[00163] Mass labels suitable for use in various embodiments herein include, by way of non-limiting example, D-2-anthranilic acid, D-2-aminopyridine, D-methyl iodide, ^{13}C methyl iodide, deuterated-pyridyl-amine, D-biotin or the like. The mass labels can be attached by permethylation or reductive amination by any method that is known to those of skill in the art.

[00164] Affinity labels suitable for use in various embodiments herein include, by way of non-limiting example, biotin and derivatives.

[00165] Radio labels suitable for use in various embodiments herein include, by way of non-limiting example, sodium borotritide (NaB^3H_4), or the like.

[00166] Chromophores suitable for use in various embodiments herein include, by way of non-limiting example, 4-amino-1,1'-azobenzene, 4'-N,N-dimethylamino-4-aminoazobenzene, aminoazobenzene, diaminoazobenzene, Direct Red 16, CI Acid Red 57, CI Acid Blue 45, CI Acid Blue 22, CL Mordant Brown 13, CI Direct Orange 75, or the like. The chromophores may be labeled by any method that is known to those of skill in the art, such as reductive amination with the chromophore and sodium cyanoborohydride.

[00167] In some embodiments, the detectable label is an antibody. In specific embodiments, the antibody is attached to a detectable compound, such as mass labels, radiolabels, chromophores, fluorescent labels, or the like. In some embodiments, antibodies are

themselves detected and/or are detectable in various manners, e.g., as a chromophore, a fluorophore, or the like; or with a probe (e.g., using dot blot techniques, immune-detection techniques, or the like).

Purification Methods

[00168] In some embodiments, the processes described herein comprises further treatment steps of the test and/or control samples. For example, in some embodiments, the samples are homogenized and/or purified. In specific embodiments homogenization is achieved in any suitable manner including, by way of non-limiting example, with a basic solution (e.g., 0.1 N NaOH), sonication, tissue grinding or other chemical agents).

[00169] In certain embodiments, samples, including test samples and/or control samples, described herein are optionally purified prior to glycosaminoglycan processing (e.g., lyase treatment) and/or characterization. Test samples and/or control samples (i.e., one or more or all of the glycans found therein) are optionally purified using any suitable purification technique. Test samples and/or control samples are optionally purified at any suitable point in a process described herein, including before or after tagging of the glycans founds within the sample. In certain embodiments, purification techniques include centrifugation, electrophoresis, chromatography (e.g., silica gel or alumina column chromatography), gas chromatography, high performance liquid chromatography (HPLC) (e.g., reverse phase HPLC on chiral or achiral columns), thin layer chromatography, ion exchange chromatography, gel chromatography (e.g., gel filtration or permeation or size exclusion chromatography, gel electrophoresis), molecular sieve chromatography, affinity chromatography, size exclusion, filtration (e.g. through a florisil or activated charcoal plug), precipitation, osmosis, recrystallization, fluorous phase purification, distillation, extraction, chromatofocusing, supercritical fluid extraction, preparative flash chromatography (e.g., flash chromatography using a UV-Vis detector and/or a mass spectrometer (e.g., using the Biotage® suite of products) or the like.

[00170] In some embodiments, glycosaminoglycans are naturally found attached to a core protein (together forming a proteoglycan). In some embodiments, provided herein are purification processes of separating glycosaminoglycan fragments from proteoglycans prior to processing the glycosaminoglycans for detection.

Therapeutic Methods

[00171] Provided in certain embodiments are methods of treating disorders associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans (GAGs), the methods comprising:

a. administering an agent for treating MPS (e.g., an agent that modulates (e.g., promotes and/or inhibits) glycosaminoglycan biosynthesis and/or degradation) to an individual in need thereof;
b. monitoring the accumulation of glycosaminoglycans in the individual using any process described herein for detecting or quantifying the amount of oligosaccharides (e.g., mono-, di- and/or tri-saccharides, such as glycosaminoglycan oligosaccharides, including keratan sulfate fragments) present in a lyase digested biological sample (e.g., urine, serum, plasma, PBMC or CSF sample) according to any process described herein.

[00172] Provided in further or alternative embodiments are methods of monitoring the treatment of disorders associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans, the methods comprising:

a. following administration of an agent for treating MPS (e.g., an agent that modulates (e.g., promotes and/or inhibits) glycosaminoglycan biosynthesis and/or degradation) to an individual in need thereof, using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more C4-C5 non-reducing end saturated oligosaccharides present in a transformed biological sample that has been prepared by: treating a population of glycosaminoglycans, in or isolated from a biological sample taken from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more C4-C5 non-reducing end saturated oligosaccharide;
b. displaying or recording the presence of or a measure of a population of one or more oligosaccharide.

[00173] In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s). In some embodiments, the agent is

administered one or more times. In certain embodiments, the agent is administered multiple times. In some embodiments, the agent is administered in a loading dose one or more times (e.g., in a loading dosing schedule) and subsequently administered in a maintenance dose (e.g., in a maintenance dosing schedule, such as three times a day, twice a day, once a day, once every two days, once every three days, once every four days, once a week, or the like). In some embodiments, when glycosaminoglycan oligosaccharide accumulation begins to increase or accelerate, the dose is optionally adjusted (e.g., the maintenance dose is increased, or an additional loading dose or dosing schedule is utilized).

[00174] In some embodiments, monitoring the accumulation of glycosaminoglycans comprises repeating the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the step is repeated at periodic intervals (e.g., every day, every other day, every 2 days, every 3 days, every 4 days, every week, every month, every 3 months, quarterly, every 6 months, yearly, or the like), at regular times following a dose (e.g., 4 hours after a administration of the agent, 6 hours after administration of the agent, 8 hours after administration of the agent, 12 hours after administration of the agent, or the like), prior to administration of the dose (e.g., immediately prior to administration of the agent, 2 hours prior to administration of the agent, or the like), or any other monitoring schedule.

[00175] In some embodiments, the monitoring of the accumulation of glycosaminoglycans is conducted over a period of time, e.g., over a week, two weeks, a month, two months, three months, six months, a year, or the like. In some embodiments, the method for quantifying the amount of one or more oligosaccharides in a lyase digested biological sample (e.g., urine, serum, plasma, PBMC, or CSF) comprises detecting and/or measuring (e.g., with an analytical device), one or more oligosaccharides within the lyase digested biological sample from the individual after the biological sample obtained from the individual has been treated with one or more glycosaminoglycan lyases. In certain embodiments, such glycosaminoglycan lyases are suitable for preparing mono-, di- and/or tri-saccharides from the glycosaminoglycans present in the

biological sample obtained from the individual. In certain instances, the reducing end of a representative portion of the one or more oligosaccharides in the transformed biological sample is tagged with any suitable detectable label (e.g., a mass label, a radio label, a fluorescent label, a chromophore label, affinity label, an antibody). In some embodiments, the process comprises displaying or recording such a characterization of the population of oligosaccharides and/or tagged oligosaccharides.

[00176] In some embodiments, the agent that modulates glycosaminoglycan biosynthesis includes glycosaminoglycan accumulation inhibitors, agents that promote glycosaminoglycan degradation, agents that activate enzymes that degrade glycosaminoglycans, agents that inhibit biosynthesis of glycosaminoglycans, or the like. In some embodiments, the agent that modulates glycosaminoglycan biosynthesis is an agent that selectively modulates chondroitin sulfate biosynthesis, an agent that selectively modulates dermatan sulfate biosynthesis, an agent that selectively modulates keratan sulfate biosynthesis, or a combination thereof.

[00177] In some instances, the detection and/or the quantification of the identity and/or amount of oligosaccharides present in a biological sample is used to identify and/or diagnose a disorder associated with abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans in an individual suspected of having such a disorder, e.g., MPS IV.

[00178] In some instances, the detection and/or the quantification of the identity and/or amount of oligosaccharides present in the biological sample is used to monitor severity and course of the disease in an individual diagnosed with or suspected of having a disorder associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans, e.g., MPS IV. In some instances, the detection and/or the quantification of the identity and/or amount of oligosaccharides present in the biological sample is used to calculate the administered dose of an agent that modulates (e.g., promotes and/or inhibits) glycosaminoglycan biosynthesis and/or degradation.

[00179] In certain instances, wherein following administration of a selected dose of a an agent that modulates (e.g., promotes and/or inhibits) glycosaminoglycan biosynthesis and/or degradation, an individual's condition does not improve, the detection and/or the quantification of the identity and/or amount of oligosaccharides present in a biological sample provides for a treatment regimen to be modified depending on the severity and course of the disease, disorder

or condition, previous therapy, the individual's health status and response to the drugs, and the judgment of the treating physician.

[00180] In certain embodiments, monitoring the accumulation of glycosaminoglycans in the individual comprises detecting or quantifying the amount of an oligosaccharide (or one or more oligosaccharides) in a sample obtained from the individual (e.g., according to any method described herein) to obtain a first accumulation result (e.g., an initial reading before treatment has begun, or at any other time) and a second accumulation result that is subsequent to obtaining the first result. In some embodiments, the second result is compared to the first result to determine if the treatment is effectively reducing, maintaining, or reducing the rate of increasing the oligosaccharide levels in a substantially identically obtained sample from the individual being treated. In certain embodiments, depending on the difference between the first and second results, the treatment can be altered, e.g., to increase or decrease the amount of agent administered; to substitute the therapeutic agent with an alternative therapeutic agent; or the like. In certain embodiments, the dose of the therapeutic agent is decreased to a maintenance level (e.g., if the oligosaccharide level has been reduced sufficiently); further monitoring of oligosaccharide levels is optional in such situation, e.g., to ensure that reduced or maintained levels of oligosaccharide (e.g., glycosaminoglycan oligosaccharide(s)) are achieved.

[00181] Alternatively, provided herein is a method of detecting response to therapy in an individual or a method of predicting response to therapy in an individual comprising:

a. administering an agent for treating MPS IV (e.g., an agent that modulates (e.g., promotes and/or inhibits) glycosaminoglycan biosynthesis and/or degradation) to a plurality of cells from an individual in need thereof (e.g., a plurality of fibroblasts, serum, plasma, PBMC or CSF cells from a human suffering from MPS IV);

b. monitoring the accumulation of glycosaminoglycans in the plurality of cells using any process described herein for detecting or quantifying the amount of oligosaccharides (e.g., mono-, di- and/or tri-saccharides, such as glycosaminoglycan oligosaccharides, including keratan sulfate, chondroitin sulfate, or dermatan sulfate fragments) present in a lyase digested biological sample from the plurality of cells according to any process described herein.

[00182] In specific embodiments, the oligosaccharide(s) detected or measured is one or more C4-C5 non-reducing end saturated oligosaccharide(s). It is to be understood that a plurality of cells from an individual includes cells that are directly taken from the individual, and/or cells that are taken from an individual followed by culturing to expand the population thereof.

[00183] In some embodiments, monitoring the accumulation of glycosaminoglycans comprises repeating the step of: using an analytical instrument to detect the presence of and/or measure the amount of a population of one or more oligosaccharides present in a transformed biological sample that has been prepared by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan lyase to transform the glycosaminoglycans into the population of the one or more oligosaccharide. In specific embodiments, the step is repeated at periodic intervals (e.g., every day, every other day, every 2 days, every 3 days, every 4 days, every week, or the like), or any other monitoring schedule.

[00184] As discussed in Example 6 below and shown in Figure 6, the level of 6-sulfated GalNAc significantly reduced post treatment with elosulfase alfa as compared with pre-treatment level of 6-sulfated GalNAc for each patient. As such, the level of 6-sulfated GalNAc can also be used to monitor a MPS IVA patient's response to a treatment.

[00185] In other embodiments, provided herein is a process of monitoring the efficacy of a treatment to an individual with MPS IVA, comprising:

a. administering a treatment to the individual with MPS IVA;

b. obtaining a biological sample from the individual with MPS IVA;

c. treating a population of chondroitin sulfates or dermatan sulfates, in or isolated from the biological sample, with chondroitinase ABC;

d. using an analytical instrument to measure the amount of 6-sulfated GalNAc produced and displaying or recording a measure of 6-sulfated GalNAc;

e. comparing the level of 6-sulfated GalNAc in the biological sample with a reference level of 6-sulfated GalNAc; and

f. determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than the reference level of 6-sulfated GalNAc,

wherein the reference level of the biomarker is determined by using a control sample obtained from the same individual prior to administering the treatment agent.

[00186] In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 90% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 80% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 70% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 60% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 50% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 40% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 30% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 20% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 10% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 5% of the reference level of 6-sulfated GalNAc. In some embodiments, the method provided herein comprises determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 3% of the reference level of 6-sulfated GalNAc.

[00187] In some embodiments, the treatment comprises administering elosulfase alfa. In some embodiments, the treatment is selected from a group consisting of enzyme replacement therapy (ERT), bone marrow transplantation (BMT), and umbilical cord blood transplantation (UCBT). Other treatments to MPS IVA known to one skilled in the art are also included in the present disclosure.

Disorders

[00188] Disorders associated with the abnormal degradation, biosynthesis and/or accumulation of glycosaminoglycans useful in the treatment and diagnostic methods and processes described herein include disorders wherein accumulation of glycosaminoglycans and/or fragments thereof can be detected in a biological sample taken from an individual suffering from such a disorder. As discussed herein, disorders associated with abnormal glycosaminoglycan degradation, biosynthesis, and/or accumulation include e.g., lysosomal storage diseases. In specific embodiments, a lysosomal storage disease is mucopolysaccharidosis (MPS). In some embodiments, a mucopolysaccharidosis (MPS) is MPS IV. In a specific embodiment, the disorder is MPS IVA. In another specific embodiment, the disorder is MPS IVB.

[00189] In some instances, a lysosomal storage disease is caused by abnormal glycosaminoglycan degradation. In some instances, a lysosomal storage disease causes an accumulation of keratan sulfate (e.g., MPS IVA) and is caused by a galactose 6-sulfatase deficiency. In some instances, a lysosomal storage disease causes an accumulation of keratan sulfate (e.g., MPS IVB) and is caused by an N-acetylglactosamine 4-sulfatase deficiency.

[00190] In some embodiments, a disorder associated with abnormal glycosaminoglycan degradation, biosynthesis and/or accumulation is undesired angiogenesis (e.g., angiogenesis associated with cancer, diabetic blindness, age-related macular degeneration, rheumatoid arthritis, or psoriasis), insufficient angiogenesis (e.g., coronary artery disease, stroke, or delayed wound healing), amyloidosis, a spinal cord injury, hypertriglyceridemia, inflammation, or a wound.

[00191] In some instances, amyloidosis is present in various diseases including, e.g., Alzheimer's disease, Parkinson's disease, type-2 diabetes, Huntington's disease, spongiform

encephalopathies (Creutzfeld-Jakob, Kuru, Mad Cow), diabetic amyloidosis, rheumatoid arthritis, juvenile chronic arthritis, Ankylosing spondylitis, psoriasis, psoriatic arthritis, adult still disease, Becet syndrome, famalial Mediterranean fever, Crohn's disease, leprosy, osteomyelitis, tuberculosis, chronic bronciectasis, Castleman disease, Hodgkin's disease, renal cell carcinoma, carcinoma of the gut, lung or urogenital tract. In some instances, the Alzheimer's disease is associated with changes in the content and structure of one or more glycosaminoglycan (e.g., keratan sulfate).

[00192] In some embodiments, disorders associated with abnormal glycosaminoglycan accumulation include disorders associated with abnormal biosynthesis (e.g., polymerization and/or sulfation) of glycosaminoglycans. In certain instances, the abnormal biosynthesis of glycosaminoglycans results in glycosaminoglycans that are not readily degraded by normal glycosaminoglycan degrading enzymes. In some instances, disorders associated with abnormal glycosaminoglycan biosynthesis include osteoarthritis. In certain instances, osteoarthritis is associated with changes in sulfation of chondroitin sulfate, changes in length of chondroitin sulfate, changes in expression levels of chondroitin sulfate, or any combination of thereof. In some instances, osteoarthritis is associated abnormal chondroitin sulfate sulfotransferase. In certain instances, the osteoarthritis is associated with changes in sulfation of dermatan sulfate, changes in length of dermatan sulfate, changes in expression levels of dermatan sulfate, or any combination of thereof. In certain instances, the osteoarthritis is associated with changes in sulfation of keratan sulfate, changes in length of keratan sulfate, changes in expression levels of keratan sulfate, or any combination of thereof.

[00193] In some embodiments, a disorder associated with abnormal glycosaminoglycan degradation, biosynthesis and/or accumulation is macular corneal dystrophy. In some instances, macular corneal dystrophy is associated with low amounts of keratan sulfate. In more specific embodiments, the keratan sulfate levels are due to failure to initiate keratan sulfate synthesis, polymerize the keratan sulfate chain length, or any combination thereof.

[00194] In some embodiments, a disorder associated with abnormal glycosaminoglycan degradation, biosynthesis and/or accumulation is a cancer. In certain embodiments, the cancer is breast cancer, ovarian cancer, colorectal cancer, cervical cancer, pancreatic cancer, gastric cancer, esophageal cancer, head and neck cancer, hepatocellular cancer, prostate cancer,

melanoma, osteosarcoma, endometrial cancer, multiple myeloma, gastric cancer, lung cancer, glioma, kidney cancer, bladder cancer, thyroid cancer, neuroblastoma, or non-Hodgkin lymphoma.

[00195] In certain instances, cancer is associated with abnormal chondroitin sulfate sulfation. In more specific embodiments, the abnormal chondroitin sulfate sulfation is associated with lung cancer. In some instances, the chondroitin sulfate sulfation is increased in certain cancers. In certain instances, the abnormal chondroitin sulfate sulfation is caused by abnormal chondroitin sulfate sulfotransferase function. In some instances, increased production of chondroitin sulfate is associated in breast cancer, melanoma, and transformed fibroblasts.

[00196] In certain instances, cancer is associated with dermatan sulfate epimerase expression. In some instances, the dermatan sulfate epimerase expression is increased in squamous cell carcinoma, glioma, gynecological cancer, pancreatic cancer, colorectal carcinoma, and prostate cancer. In certain instances, the cancer is associated with accumulation of dermatan sulfate levels. In some instances, the dermatan sulfate levels are increased in pancreatic cancer.

[00197] In certain instances, cancer is associated with abnormal keratan sulfate sulfation. In some instances, the abnormal keratan sulfate sulfation is associated with glioblastomas. In certain instances, abnormal keratan sulfate sulfation is caused by abnormal keratan sulfate sulfotransferase function. In some instances, keratan sulfate expression is increased in glioblastomas.

Drug Screens

[00198] Provided in certain embodiments herein is a process for identifying an agent that inhibits the accumulation of glycosaminoglycans in a cell, the process comprising:

a. contacting a plurality of mammalian cells with a compound, the plurality of mammalian cells being of a cell line that accumulates an abnormal amount of glycosaminoglycans;

b. incubating the mammalian cells with the compound;

c. optionally transforming the mammalian cells e.g., by isolating a population of glycosaminoglycans from the cells (e.g., using any suitable method described herein);

d. contacting the mammalian cells and/or the isolated population of glycosaminoglycans from step (c) with a glycosaminoglycan lyase (e.g., keratanase or chondroitinase);

e. purifying a sub-population of oligosaccharides from step (d) (e.g., using any suitable method described herein);

f. detecting the presence of and/or measuring the amount of one or more oligosaccharides present in the sub-population (e.g., using LC-MS or GC-MS); and

g. displaying or recording a characterization of the sub-population of one or more oligosaccharides.

[00199] In certain instances, the mammalian cells are optionally transformed by e.g., tagging a population of glycosaminoglycans on and/or in the cells with a detectable label. In some instances, the mammalian cells are optionally transformed by e.g., isolating a population of glycosaminoglycans on and/or in the cells using any suitable purification technique described herein.

[00200] In certain embodiments, the cell is present in an individual (e.g., a human or other mammal) and is incubated at body temperature. In some embodiments, the cell line that accumulates an abnormal amount of glycosaminoglycans being a mucopolysaccharidosis (MPS) cell line (e.g., a human MPS cell line). In more specific embodiments, the MPS cell line is a cell line for MPS IVA, MPS IVB, or a combination thereof. In more specific embodiments, the MPS cell line is a cell line for MPS IVA. In more specific embodiments, the MPS cell line is a cell line for MPS IVB. In some embodiments, the human MPS cell line is present in a human or other mammal. In some embodiments, inhibitors of the accumulation of glycosaminoglycans are compounds that reduce the rate of accumulation of glycosaminoglycans in the cell, and/or agents that reduce the total amount of glycosaminoglycans accumulated in the cell (i.e., diminish the amount of glycosaminoglycan that has been accumulated in the cell). Agents that are optionally tested for the screening process described herein include any compound such as, by way of non-limiting example, a polynucleotide (e.g., siRNA), a polypeptide, or a small molecule compound (e.g., having a molecular weight of less than 2,000 g/mol).

Examples

[00201] The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are intended to be merely illustrative.

[00202] Example 1 - Purification: The biological sample (cells, tissue, blood, serum, or the like) is homogenized and solublized in 0.1 - 1.0 N NaOH (e.g., 0.1 N, 0.2 N, 0.3 N, 0.4 N, 0.5 N, 0.6 N, 0.7 N, 0.8 N, 0.9 N, or 1.0 N) or acetic acid and then neutralized with acetic acid or NaOH. Next a small sample is taken to measure protein content of the sample using standard methods. 0.01 - 0.5 mg/mL (0.01 mg/mL, 0.07 mg/mL, 0.12 mg/mL, 0.17 mg/mL, 0.22 mg/mL, 0.27 mg/mL, 0.32 mg/mL, 0.37 mg/mL, 0.42 mg/mL, or 0.5 mg/mL) protease (trypsin, chymotrypsin, pepsin, pronase, papain, or elastase) is treated in 0.1 – 0.5 M (e.g., 0.1 M, 0.16 M, 0.23 M, 0.32 M, 0.39 M, 0.44 M, or 0.5 M) NaCl, 0.01 – 0.1 M (e.g., 0.01 M, 0.02 M, 0.04 M, 0.06 M, 0.08 M, 0.1 M) NaOAc, at pH 5.5 – 7.5 (e.g., 5.5, 6.0, 6.5, 7.0, or 7.5) and 25 - 40 C (e.g., 25 C, 30 C, 35 C, or 40 C) for 1 – 24 hours (e.g., 1 h, 2 h, 4 h, 6 h, 8h, 12 h, 18 h, 24 h). The sample is diluted to reduce the ionic strength and loaded onto an ion exchange column in 5 – 100 mM (e.g., 5 mM, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 75 mM, 80 mM, 90 mM, 95 mM, 100 mM) NaOAc pH 5 – 7 with 0 – 300 mM NaCl. After washing, the bound glycosaminoglycans are eluted with 5 – 100 mM NaOAc pH 5 – 7 (e.g., 5, 5.5, 6, 6.5, 7) with 0.8 - 3 M (e.g., 0.8 M, 1 M, 1.2 M, 1.4 M, 1.6 M, 1.8 M, 2 M, 2.5 M, or 3 M) NaCl. The eluted glycans are then concentrated and desalted by ethanol precipitation, size exclusion, or other methods. The purified glycans are dried for further analysis.

[00203] Example 2 – Digestion: Lyase digestion: The purified glycans are resuspended in 10 – 300 mM sodium acetate, tris, phosphate, or other suitable buffer, 0.02 – 1 mM (e.g., 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1) calcium acetate, pH 5 – 8 (e.g., 5, 5.5, 6, 6.5, 7, 7.5, or 8), and digested with the digesting enzymes provided herein (e.g., 0.0.15 - 1.5 milliunits of each in 100-ul reactions) at 25 to 37 °C for 1 to 24 hours.

[00204] Example 3 – Tagging: Dried glycan sample is re-suspended in 2 – 100 µL 0.003 – 0.1 M (e.g., 0.003 M, 0.003 M, 0.03 M, 0.06 M, 0.1 M) AB, AA, AMAC, or Bodipy dye and incubated at room temperature for 1 – 120 minutes (e.g., 1-10 min, 10-15 min, 15-20 min, 20-25 min, 25-30 min, 30-40 min, 40-50 min, 50-60 min, 60-90 min, 90-120 min). Next, the reaction is

initiated with 2 – 100 μL (2 μL, 5 μL, 10 μL, 15 μL, 20 μL, 25 μL, 30 μL, 40 μL, 50 μL, 60 μL, 70 μL, 80 μL, 90 μL, or 100 μL) 1 M $NaCNBH_4$ and the reaction is allowed to proceed at 25 – 100 C. (e.g., 25 C, 30 C, 35 C, 40 C, 50 C, 60 C, 70 C, 80 C, 90 C, 100 C).

[00205] Example 4 – Detecting: HPLC separation of tagged saccharides was performed utilizing the following conditions:

- Column types: 130A BEH particle Phenyl (1.7, 2.5, 3.5, 5, or 10 uM particle size), 130A BEH particle C18 (1.7, 2.5, 3.5, 5, or 10 uM particle size), HSS particle C18 (1.8, 3.5, or 5 uM particle size), or 300A BEH particle C18 (1.7, 3.5, 5, 10 uM particle size) with suitable length and internal diameter.
- Buffer Conditions:
    - A = 10mM Ammonium Acetate with 0 – 20% methanol
    - B = 100% Methanol
    - Initial Conditions: 70 – 95% A, 0 – 30% B
- Flow Rate is constant at 0.05 – 1 ml/min
- Runs a gradient down to 70 – 90% A, 10 – 30% B over 5 – 65 min.
- At 8.1 min runs a gradient to 0 - 20% A, 80 - 100% B over 5 – 20 min.
- 5 – 65 min returns to initial conditions

[00206] Fluorescently tagged oligosaccharides were detected at various elution times depending on the specific marker produced of interest.

[00207] Example 5—Analysis of Keratan Sulfate (KS) Using Keratanases: In these studies, urine samples were collected from unaffected normal individuals and individuals known to be affected with MPS VI A (Morquio A). Keratanase I or keratanase II were used to digest the samples. **Figure 2B** shows the results using keratanase I. Signal intensity peak areas for odd vs even are utilized as a measure of biomarker levels. As shown, the chromatographs indicate a significant increase in odd numbered oligosaccharides in Morquio A urine KS after digestion with Keratanase I. The ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is 1:2; while the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA is 1:5. The difference is sufficient to distinguish unaffected from affected individuals and to measure disease severity

and therapeutic efficacy. **Figure 3B** shows the results using keratanase II. In this study, an isobaric pair of tetrasaccharides was identified as a biomarker for Morquio A (MPS IVA). The two tetrasaccharides have the same mass and similar ionization potential which makes them good LC/MS analytes to evaluate the Morquio A disease state. In an unaffected normal individual, the abundance ratio of the two tetrasaccharides is approximately 1:1. In an affected individual there is an increase in 6-sulfated galactose at the NRE of KS chains and the tetrasaccharide with 6-sulfated galactose at the NRE is predominate and the abundance ratio of the two tetrasaccharides shifts as shown in **Figure 3B**. As shown, the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is 10:1; and the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA is 1:1. In addition, the ion intensity of the fully sulfated tetrasaccharide (last peak in the chromatograms) is increased in the affected individuals, which can be used as a verification of the results.

[00208] Example 6—Analysis of Chondroitin Sulfate (CS) Using Chondroitinase ABC: In these studies, urine samples were collected from unaffected normal individuals and individuals known to be affected with MPS VI A (Morquio A). Chondroitinase ABC was used to digest the samples. As shown in **Figure 4A**, the level of 6-sulfated GalNAc increases in the individuals with MPS IVA post enzyme digestion as compared with normal individuals, and as such, the level of 6-sulfated GalNAc post enzyme digestion can be used for diagnosing an individual with MPS IVA. This result is further verified in a study using adult normal individuals as compared with adult MPS IVA patients, the result of which is shown in **Figure 5**. As shown, after digestion, the level of 6-sulfated GalNAc in MPS IVA patients is more than 100-fold of that of normal individuals.

[00209] At least partially due to the increased level of 6-sulfated GalNAc in MPS IVA patients, as shown in **Figure 4B**, chondroitinase ABC digestion of CS/DS samples resulted in large increase in 6-sulfated GalNAc relative to 4-sulfated GalNAc in MPS IVA samples compared to unaffected samples. The ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc

at the non-reducing end in samples from individuals with MPS IVA is more than 10:1; while the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA is less than 1:1.

[00210] A study was also performed to determine if the level of 6-sulfated GalNAc could be used to monitor a MPS IVA patient's response to a treatment. Samples were obtained from four MPS IVA patients and a normal individual before and after treatment with VIMIZIM® (elosulfase alfa), indicated for treating MPS IVA. These samples were digested with Chondroitinase ABC, and the level of 6-sulfated GalNAc was measured for each sample. The results are shown in **Figure 6**, and as shown, the level of 6-sulfated GalNAc significantly reduced post treatment as compared with pre-treatment level for each patient. These results confirm that the level of 6-sulfated GalNAc can be used to diagnose a MPS IVA patient and to monitor a MPS IVA patient's response to a treatment.

[00211] From the foregoing, it will be appreciated that, although specific embodiments have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of what is provided herein. All of the references referred to above are incorporated herein by reference in their entireties.

WHAT IS CLAIMED IS:

1. A process for diagnosing the presence, identity, and/or severity of abnormal glycosaminoglycan accumulation in an individual, or a disorder thereof, the process comprising the steps of:

a) generating one or more biomarkers comprising of one or more non-reducing end oligosaccharides, wherein the one or more biomarkers are oligosaccharides and are generated by treating a population of glycosaminoglycans, in or isolated from a biological sample from the individual, with at least one digesting glycosaminoglycan enzyme, wherein after enzyme treatment, the amount of the one or more biomarkers in samples from individuals with abnormal glycosaminoglycan accumulation are different from the amount of the one or more biomarkers in samples from individuals with normal glycosaminoglycan, and

b) using an analytical instrument to detect the presence of and/or measure the amount of the one or more biomarkers produced and displaying or recording the presence of or a measure of a population of the one or more biomarkers,

wherein the abnormal glycosaminoglycan accumulation disorder is MPS IV;
wherein the presence of and/or measure the amount of the one or more biomarkers is utilized to determine the presence, identity, and/or severity of MPS IV; and
wherein the population of glycosaminoglycans comprises a glycosaminoglycan selected from the group consisting of keratan sulfate, chondroitin sulfate, and dermatan sulfate.

2. The process of claim 1, wherein in step (a) generating a first biomarker and a second biomarker, and wherein the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is different from the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

3. The process of claim 2, wherein the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is more than

one fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

4. The process of claim 3, wherein the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold to 100-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

5. The process of claim 3, wherein the ratio of the first biomarker to the second biomarker in the samples from individuals with abnormal glycosaminoglycan accumulation is 2-fold to 20-fold of the ratio of the first biomarker to the second biomarker in the samples from individuals with normal glycosaminoglycan.

6. The process of any one of claims 1-5, wherein the abnormal glycosaminoglycan accumulation disorder is MPS IVA.

7. The process of any one of claims 1-5, wherein the abnormal glycosaminoglycan accumulation disorder is MPS IVB.

8. The process of any one of claims 1-7, wherein the at least one digesting glycosaminoglycan enzyme is one or more non-lyase.

9. The process of any one of claims 1-7, wherein the at least one digesting glycosaminoglycan enzyme is one or more chondroitin sulfate digesting lyase, one or more dermatan sulfate digesting lyase, one or more keratanase, or a combination thereof.

10. The process of claim 9, wherein the at least one digesting glycosaminoglycan enzyme is selected from the group consisting of keratanase I and keratanase II.

11. The process of claim 9, wherein the at least one digesting glycosaminoglycan enzyme is selected from the gourp consisting of chrondroitinase ABC, chrondroitinase B, and chrondroitinase AC.

12. The process of claim 1, wherein the abnormal glycosaminoglycan accumulation is abnormal keratan sulfate accumulation.

13. The process of claim 1, wherein the abnormal glycosaminoglycan accumulation is abnormal chondroitin sulfate accumulation.

14. The process of claim 1, wherein the abnormal glycosaminoglycan accumulation is abnormal dermatan sulfate accumulation.

15. The process of any one of claims 1-14, further comprising purifying transformed biological sample using chromatography or electrophoresis.

16. The process of claim 15, wherein the chromatography is high performance liquid chromatography (HPLC), gas chromatography (GC), column chromatography, affinity chromatography, or thin layer chromatography (TLC).

17. The process of any one of claims 1-16, wherein the oligosaccharides are detected using mass spectrometry.

18. The process of any one of claims 1-17, wherein the process of preparing transformed biological sample further comprises tagging the reducing end of a representative portion of the one or more oligosaccharides in the transformed biological sample with a detectable label.

19. The process of claim 18, wherein the detectable label is a mass label, a radio label, a fluorescent label, a chromophore label, or affinity label.

20. The process of claim 19, wherein the tagged portion of the one or more oligosaccharides is detected or measured using UV-Vis spectroscopy, IR spectroscopy, mass spectrometry, or a combination thereof.

21. The process of claim 1, wherein the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzyme comprises keratan sulfate; wherein the at least one digesting glycosaminoglycan enzyme comprise keratanase I; wherein a first biomarker and a

second biomarker are generated; and wherein the abnormal glycosaminoglycan accumulation disorder is MPS IVA.

22. The process of claim 21, wherein the first biomarker is odd numbered oligosaccharide, and the second biomarker is even numbered oligosaccharide.

23. The process of claim 22, wherein the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is higher than the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA.

24. The process of claim 22, wherein the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is 2-fold to 20-fold of the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA.

25. The process of claim 22, wherein the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals with MPS IVA is 1:2; and the ratio of the amount of the odd numbered oligosaccharides to the amount of the even numbered oligosaccharides in samples from individuals without MPS IVA is 1:5.

26. The process of claim 1, wherein the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzyme comprises keratan sulfate; wherein the at least one digesting glycosaminoglycan enzyme comprise keratanase II; wherein a first biomarker and a second biomarker are generated; and wherein the abnormal glycosaminoglycan accumulation disorder is MPS IVA.

27. The process of claim 26, wherein the first biomarker is oligosaccharides with sulfated galactose at the non-reducing end; and the second biomarker is oligosaccharides with unsulfated galactose at the non-reducing end.

28. The process of claim 27, wherein the first biomarker and the second biomarker are tetrasaccharides.

29. The process of claim 27, wherein the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is higher than the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA.

30. The process of claim 29, wherein the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 1000-fold of the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA.

31. The process of claim 29, wherein the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals with MPS IVA is 10:1; and the ratio of the amount of the oligosaccharides with sulfated galactose at the non-reducing end to the amount of the oligosaccharides with unsulfated galactose at the non-reducing end in samples from individuals without MPS IVA is 1:1.

32. The process of claim 1, wherein the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzyme comprises chondroitin sulfate or dermatan sulfate; wherein the at least one digesting glycosaminoglycan enzyme comprises chondroitinase ABC; wherein a first biomarker and a second biomarker are generated; and wherein the abnormal glycosaminoglycan accumulation disorder is MPS IVA.

33. The process of claim 32, wherein the first biomarker is an oligosaccharide with 6-sulfated GalNAc at the non-reducing end; and the second biomarker is an oligosaccharide with 4-sulfated GalNAc at the non-reducing end.

34. The process of claim 33, wherein the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is higher than the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA.

35. The process of claim 34, wherein the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is 2-fold to 100-fold of the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA.

36. The process of claim 34, wherein the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals with MPS IVA is more than 10:1; and the ratio of the amount of the oligosaccharides with 6-sulfated GalNAc at the non-reducing end to the amount of the oligosaccharides with 4-sulfated GalNAc at the non-reducing end in samples from individuals without MPS IVA is less than 1:1.

37. The process of any one of claims 32 to 36, wherein the oligosaccharides with 6-sulfated GalNAc at the non-reducing end is 6-sulfated GalNAc, and the oligosaccharides with 4-sulfated GalNAc at the non-reducing end is 4-sulfated GalNAc.

38. The process of claim 1, wherein the population of glycosaminoglycans treated with at least one digesting glycosaminoglycan enzyme comprises chondroitin sulfate or dermatan sulfate; wherein the at least one digesting glycosaminoglycan enzyme comprises a chondroitin sulfate lyase or a dermatan sulfate lyase; wherein a biomarker is generated, the biomarker being 6-sulfated GalNAc; and wherein the abnormal glycosaminoglycan accumulation disorder is MPS IVA.

39. The process of claim 38, wherein the at least one digesting glycosaminoglycan enzyme is selected from the group consisting of chondroitinase ABC, chondroitinase B, and chondroitinase AC.

40. The process of claim 38, wherein the at least one digesting glycosaminoglycan enzyme is chondroitinase ABC.

41. The process of claim 38, further comprising diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is higher than a reference level of 6-sulfated GalNAc.

42. The process of claim 41, wherein the reference level of 6-sulfated GalNAc is determined by using a control sample obtained from an individual without MPS IVA.

43. The process of claim 41, wherein the reference level of 6-sulfated GalNAc is determined by using a control sample obtained from the same individual, wherein the control sample is not digested with the at least one digesting glycosaminoglycan enzyme.

44. The process of claim 41, wherein diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is 2-fold to 2000-fold of the reference level of 6-sulfated GalNAc.

45. The process of claim 41, wherein diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 20-fold of the reference level of 6-sulfated GalNAc.

46. The process of claim 41, wherein diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 50-fold of the reference level of 6-sulfated GalNAc.

47. The process of claim 41, wherein diagnosing the individual as having MPS IVA when the level of 6-sulfated GalNAc in the biological sample from the individual is more than 100-fold of the reference level of 6-sulfated GalNAc.

48. The process of any one of claims 41 to 47, further comprising administering a treatment agent to the individual diagnosed as having MPS IVA.

49. A process of monitoring the efficacy of a treatment to an individual with MPS IVA, comprising:

a) administering a treatment to the individual with MPS IVA;

b) obtaining a biological sample from the individual with MPS IVA;

c) treating a population of chondroitin sulfates or dermatan sulfates, in or isolated from the biological sample, with chondroitinase ABC;

d) using an analytical instrument to measure the amount of 6-sulfated GalNAc produced and displaying or recording a measure of 6-sulfated GalNAc;

e) comparing the level of 6-sulfated GalNAc in the biological sample with a reference level of 6-sulfated GalNAc; and

f) determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than the reference level of 6-sulfated GalNAc,

wherein the reference level of the biomarker is determined by using a control sample obtained from the same individual prior to administering the treatment agent.

50. The process of claim 49, wherein determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 90% of the reference level of 6-sulfated GalNAc.

51. The process of claim 49, wherein determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 80% of the reference level of 6-sulfated GalNAc.

52. The process of claim 49, wherein determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 50% of the reference level of 6-sulfated GalNAc.

53. The process of claim 49, wherein determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 30% of the reference level of 6-sulfated GalNAc.

54. The process of claim 49, wherein determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 20% of the reference level of 6-sulfated GalNAc.

55. The process of claim 49, wherein determining the treatment being efficacious when the level of 6-sulfated GalNAc in the biological sample is lower than 5% of the reference level of 6-sulfated GalNAc.

56. The process of claim 49, wherein the treatment comprises administering elosulfase alfa.

57. The process of claim 49, wherein the treatment is selected from a group consisting of enzyme replacement therapy (ERT), bone marrow transplantation (BMT), and umbilical cord blood transplantation (UCBT).

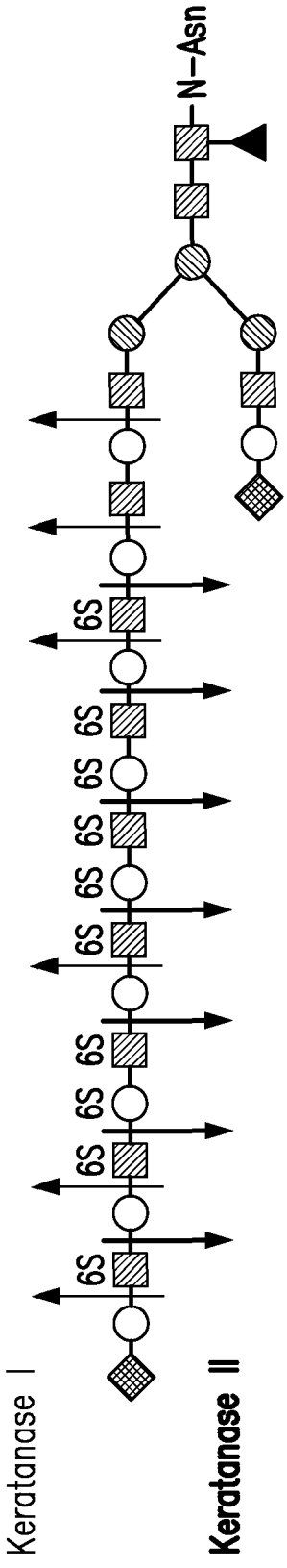


FIG. 1

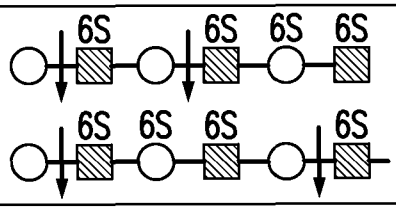
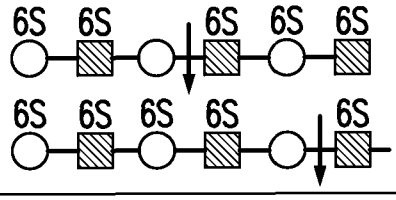
Clinical Dx	Lysosomal KS	Resulting Products
Unaffected		Predominantly even numbered oligo.s
MPS IVA		Increase in odd numbered oligo.s

FIG. 2A

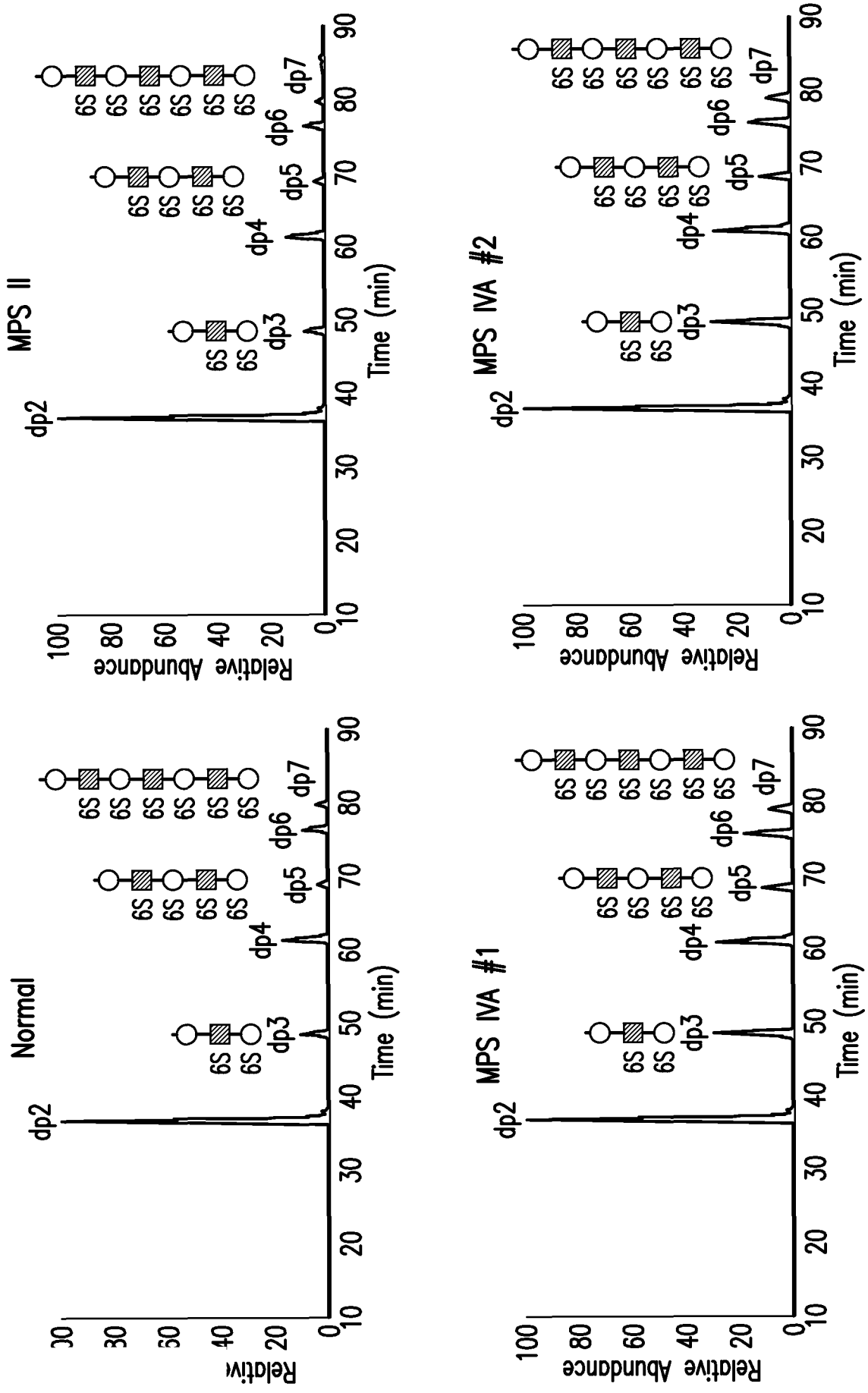

MPS II
MPS IVA #2
Normal
MPS IVA #1
Relative Abundance
Time (min)
dp2
dp3
dp4
dp5
dp6
dp7
6S

FIG. 2B

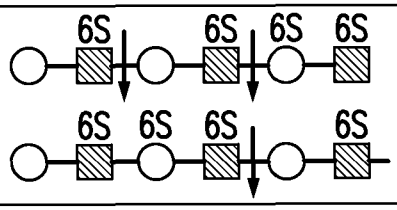
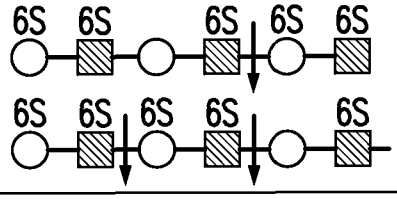
Clinical Dx	Lysosomal KS	Resulting Products
Unaffected		Similar levels of both g6A6–g0A6 and g0A6–g6A6 indicating no substantial increase in g6 NRE
MPS IVA		Contribution of g6 NRE.s will significantly increase g6A6–g0A6 levels over that of g0A6–g6A6.

FIG. 3A

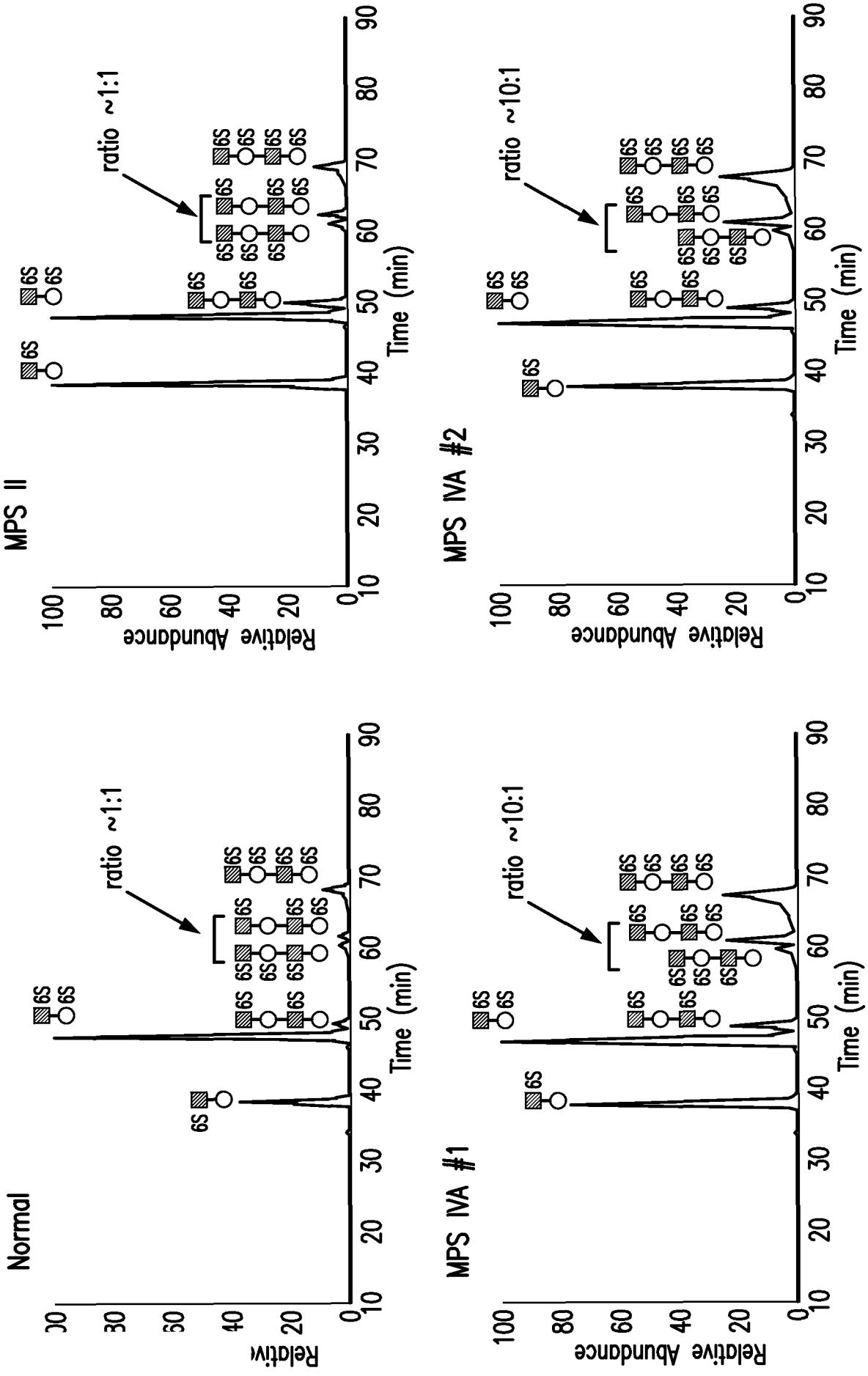

MPS II
ratio ~1:1
MPS IVA #2
ratio ~10:1
Normal
ratio ~1:1
MPS IVA #1
ratio ~10:1
Relative Abundance
Time (min)
6S

FIG. 3B

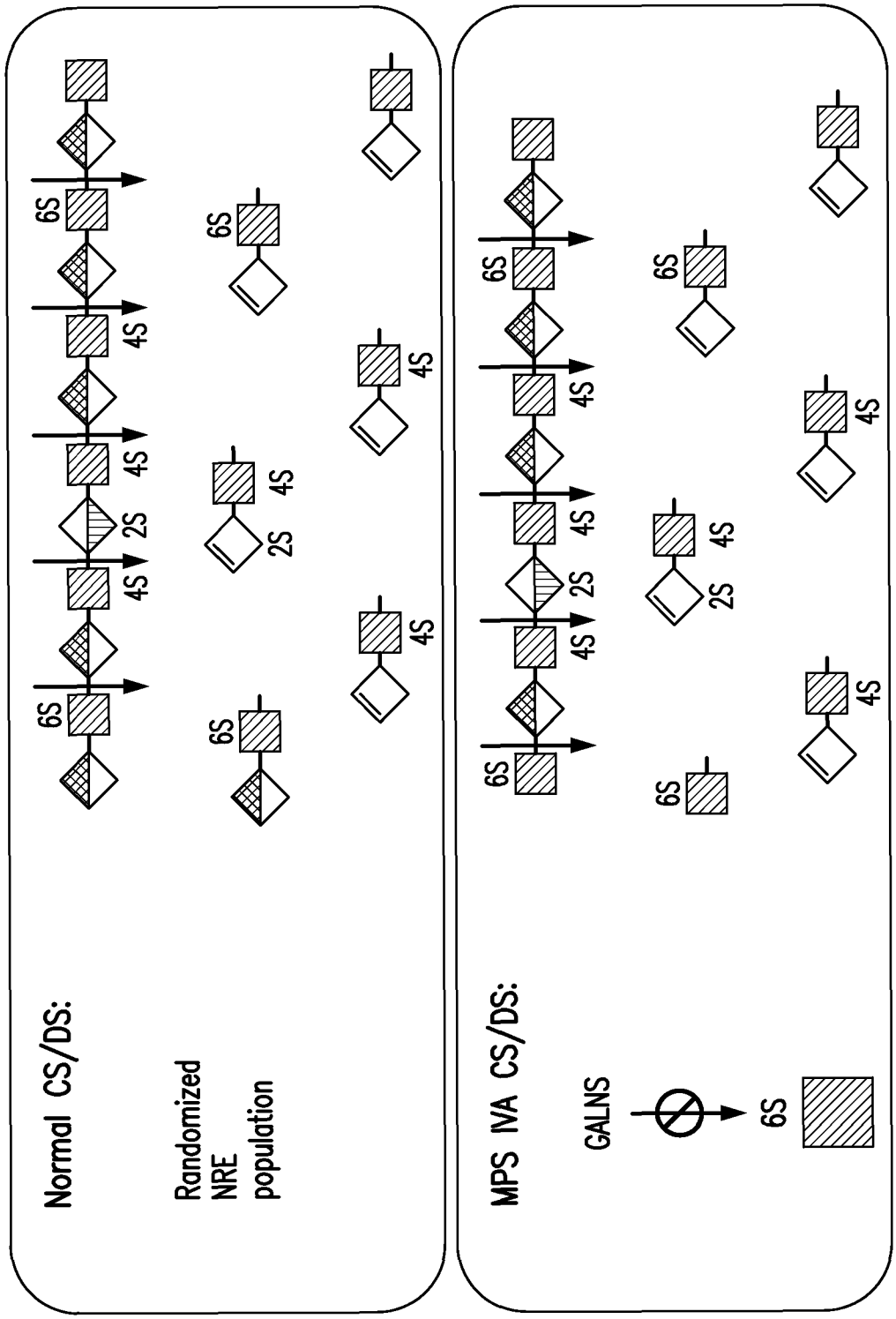

Normal CS/DS:
Randomized NRE population
MPS IVA CS/DS:
GALNS
6S
4S
2S

FIG. 4A

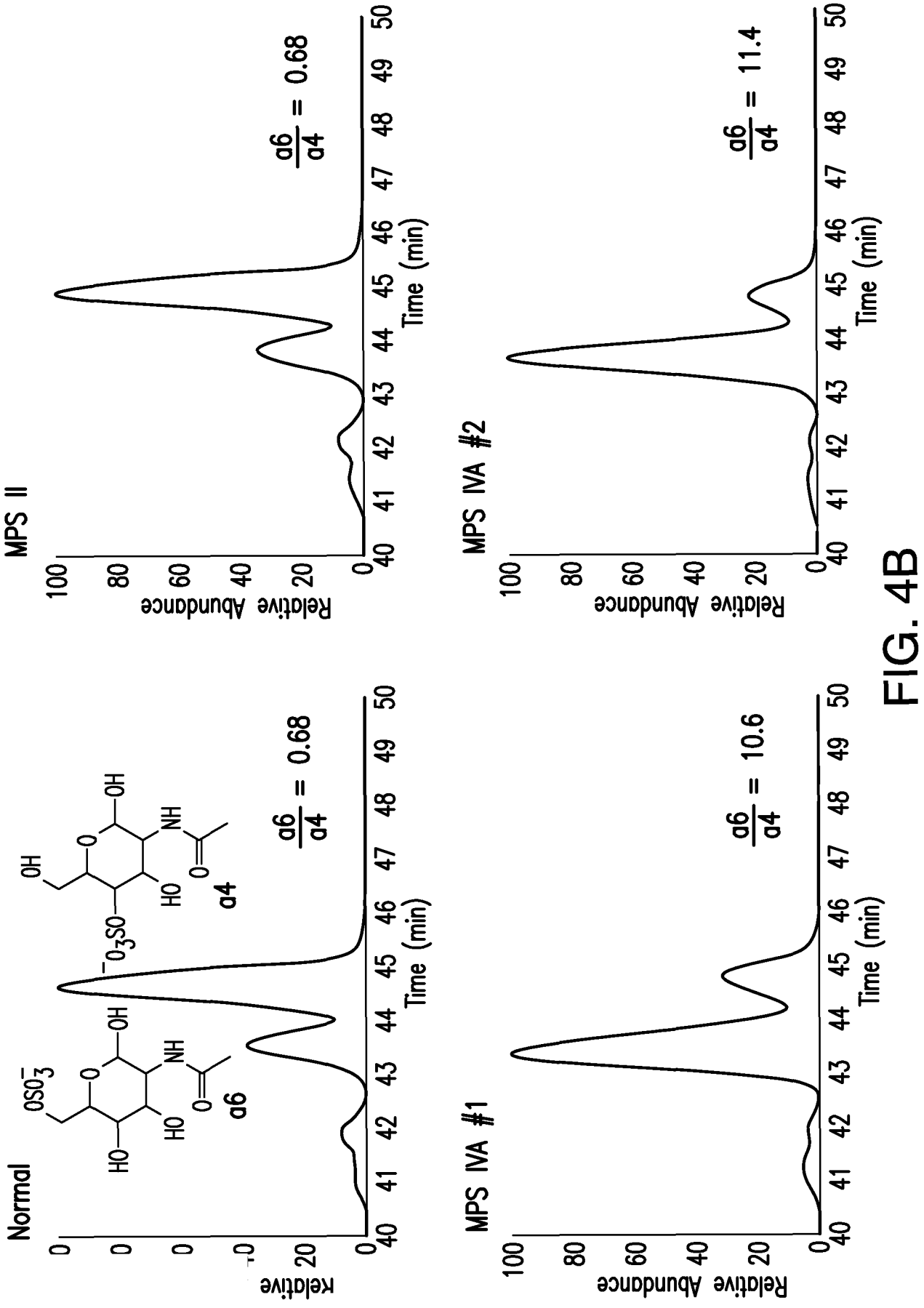

Normal
MPS II
MPS IVA #1
MPS IVA #2
Relative Abundance
Time (min)
a6/a4 = 0.68
a6/a4 = 0.68
a6/a4 = 10.6
a6/a4 = 11.4
a6
a4

FIG. 4B

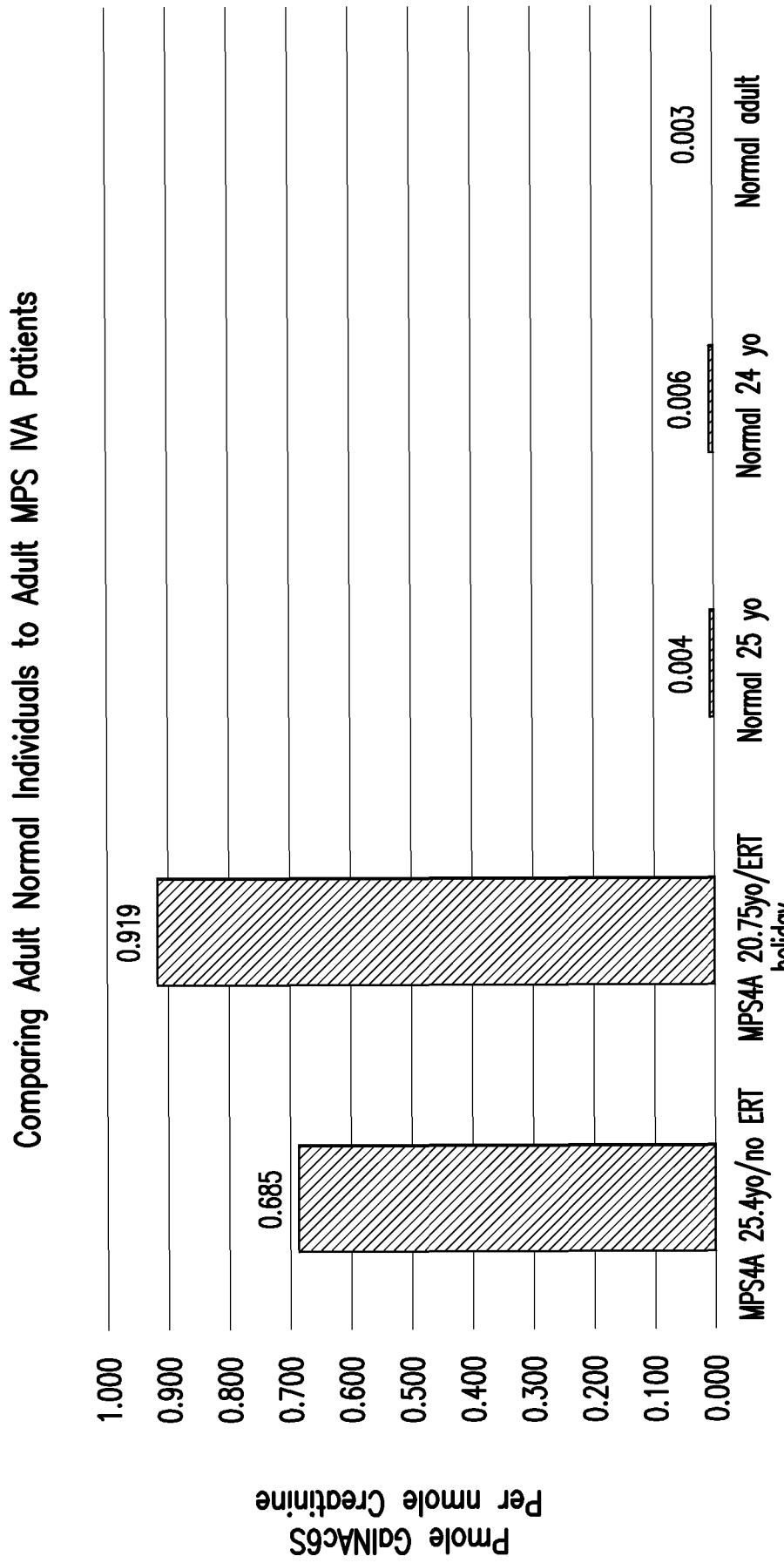

Comparing Adult Normal Individuals to Adult MPS IVA Patients
Pmole GalNAc6S
Per nmole Creatinine
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
0.685
0.919
0.004
0.006
0.003
MPS4A 25.4yo/no ERT
MPS4A 20.75yo/ERT holiday
Normal 25 yo
Normal 24 yo
Normal adult

FIG. 5

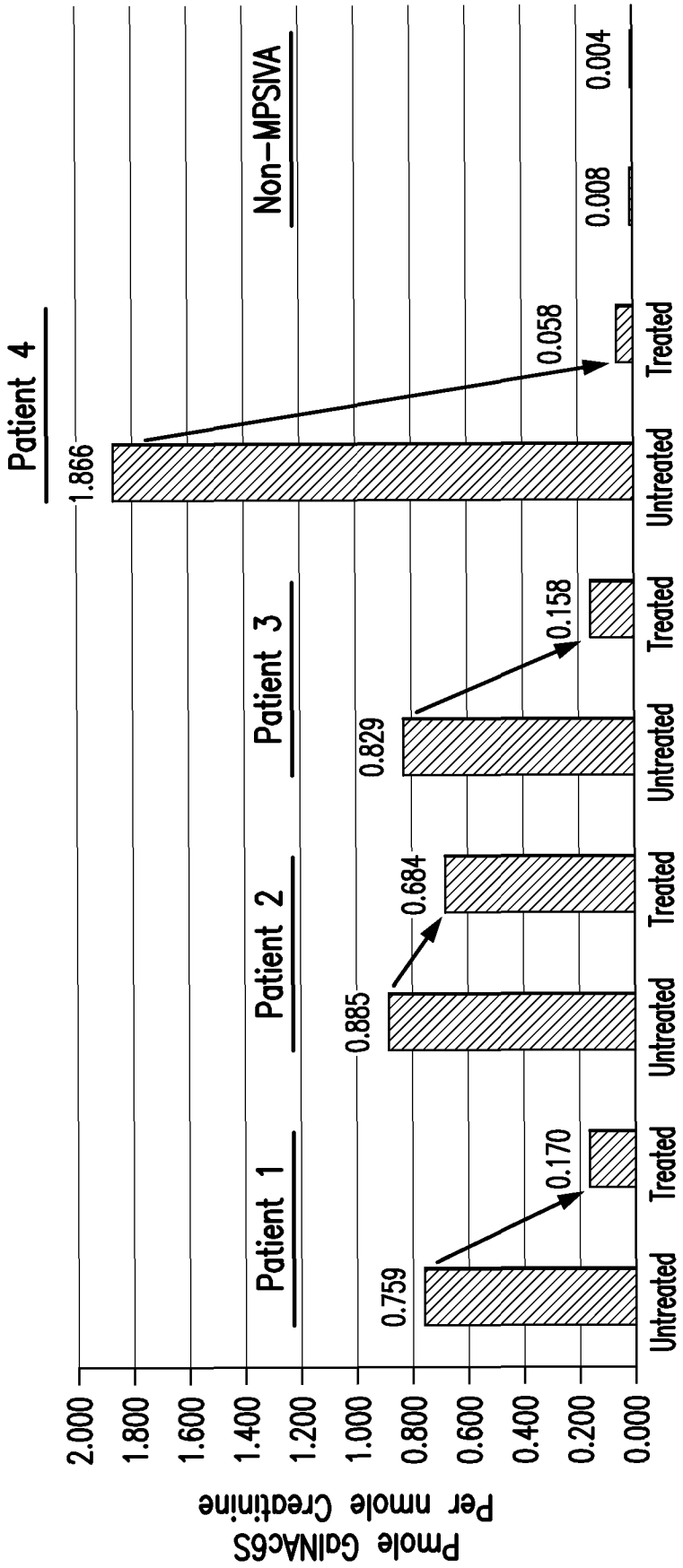

Pmole GalNAc6S
Per nmole Creatinine
2.000
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Patient 1
Patient 2
Patient 3
Patient 4
Non-MPSIVA
Untreated
Treated
0.759
0.170
0.885
0.684
0.829
0.158
1.866
0.058
0.008
0.004

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/44222

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00; C12Q 1/527 (2015.01)
CPC - C12Q 1/527; G01N 33/6893; C12Q/ 1/34
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 49/00; C12Q 1/527 (2015.01)
CPC: C12Q 1/527; G01N 33/6893; C12Q 1/34

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, RU, AT, CH, TH, BR, PH, Other Countries (INPADOC)); Google; Google Scholar; PubMed; EBSCO; glycosaminoglycan, glycan, mucopolysaccharidosis, 'MPS IV,' 'Morquio syndrome,' lyase, 'GalNAc,' chondroitin, 'dermatan sulfate,' 'keratan sulfate,' keratinase, 'elosulfase alfa'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0217056 A1 (ZACHARON PHARMACEUTICALS, INC.) August 22, 2013; paragraphs [0003], [0005], [0007]-[0010], [0016], [0026]-[0032], [0034], [0044]-[0047], [0061], [0064], [0068], [0069], [0075], [0086]-[0088], [0097], [0099], [0116], [0132], [0134], [0144], [0149], [0186], [0199]-[0204], [0219]-[0221], [0261]-[0265], [0298], [0310]; Tables 10, 12	1-3, 6/1-6/5, 7/1-7/5, 12, 13, 32-34, 37/32-37/34, 38-43, 48/41-48/43, 49, 57 ----- 4, 5, 14, 21-23, 26-28, 50-55, 56
X --- Y	US 2007/0161074 A1 (TOMATSU, S et al.) July 12, 2007; [0007], [0011], [0014], [0015], [0025]-[0038], [0079]; Tables 8, 14	1, 26, 27, 29, 30, 32 ----- 50-55
Y	KODAMA, C. et al. Liquid-Chromatographic Determination Of Urinary Glycosaminoglycans For Differential Diagnosis Of Genetic Mucopolysaccharidoses. Clin. Chem. 1986, Vol. 32, No. 1, pages 30-34.	4, 5
Y	VOLPI, N. et al. Plasmatic dermatan sulfate and chondroitin sulfate determination in mucopolysaccharidoses. J Pharm Biomed Anal. November 2013, Vol. 85, pages 40-45 (Abstract only), doi: 10.1016/j.jpba.2013.06.026; abstract.	14
Y	MINAMI, R. et al. Characterization of keratan sulfate isolated from liver affected by Morquio syndrome. Tohuku J. exp. Mep. 1983, Vol. 139, pages 321-326; pages 321-325, figure 2.	21-23
Y	WHITMAN, KM. et al. An improved method for the structural profiling of keratan sulfates: analysis of keratan sulfates from brain and ovarian tumors. Glycobiology. 1999, Vol. 9, No. 3, pages 285-291.	26-28

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2015 (13.10.2015)	13 NOV 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International

PCT/US15/44222

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-11, 15-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0216742 A1 (DEMARTINO, SE et al.) August, 22, 2013; paragraph [0396]	56