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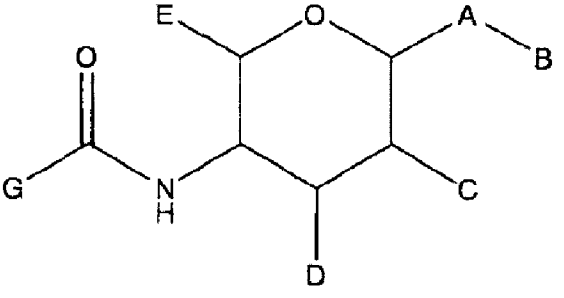
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(54) **Titre : METHODES DE TRAITEMENT DU SYNDROME NEPHROTIQUE**
(54) **Title: METHODS OF TREATING NEPHROTIC SYNDROME**



(57) **Abrégé/Abstract:**
The present disclosure provides a biochemical basis of nephrotic syndrome and provides and explanation for the observed proteinuria and other effects. As a result, the present disclosure provides method for treating and/or preventing nephrotic syndrome as well as methods of alleviating symptoms associated with nephrotic syndrome. The present disclosure further provides methods for reducing proteinuria in nephrotic syndrome and other disease states as discussed herein.

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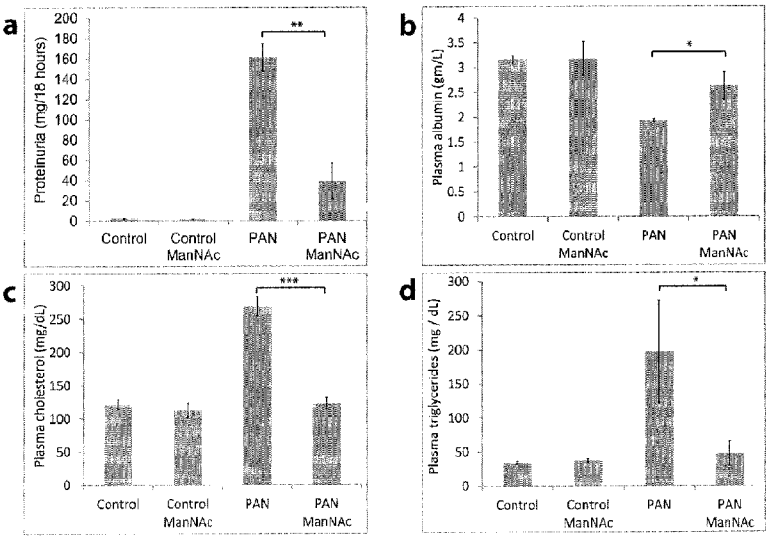


FIGURE 7

(57) Abstract: The present disclosure provides a biochemical basis of nephrotic syndrome and provides and explanation for the observed proteinuria and other effects. As a result, the present disclosure provides method for treating and/or preventing nephrotic syndrome as well as methods of alleviating symptoms associated with nephrotic syndrome. The present disclosure further provides methods for reducing proteinuria in nephrotic syndrome and other disease states as discussed herein.

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METHODS OF TREATING NEPHROTIC SYNDROME

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

Funding for the work described herein was provided by the federal government, which has certain rights in the invention.

FIELD OF THE DISCLOSURE

The present disclosure is directed to methods for the treatment and prevention of nephrotic syndrome, diabetic conditions and conditions related thereto.

BACKGROUND

Nephrotic syndrome is caused by various disorders that damage the kidneys, particularly specialized cells called podocytes and the basement membrane of the glomerulus. Diabetic nephropathy and membranous glomerulonephritis (also called membranous nephropathy) are common causes in adults, whereas minimal change disease is the most common cause in children. Characteristics of nephrotic syndrome includes loss of protein in the urine (proteinuria), hyperlipidemia (hypercholesterolemia and hypertriglyceridemia), hypoalbuminemia (low blood albumin or protein levels) and edema. Proteinuria is defined as the presence of an excess of serum proteins in the urine. Albuminuria, a specific type of proteinuria, is a pathological condition wherein albumin is present in the urine.

Podocytes (or visceral epithelial cells) are cells in the glomerular capillary loop in the kidneys. The glomerulus filters blood, holding back large molecules such as proteins, and passing through small molecules such as water, salts, and sugar, as the first step in forming urine. The long processes, or "foot projections," of the podocytes wrap around the capillaries, and leave slits between them. Blood is filtered through these slits. Kidneys affected by nephrotic syndrome have small pores in the podocytes which are large enough to permit proteins to transit, causing proteinuria.

When protein is lost in the urine, its blood concentration decreases, allowing water to move into other areas of the body, which leads to swelling known as edema. Edema is commonly observed in the feet and legs, in the belly or abdomen (ascites), and around the eyes, but can occur anywhere, especially in response to gravity. Additionally, because of this extra fluid that stays in the body, people often gain weight, experience fatigue and may find that they urinate less often.

Many conditions are categorized as nephrotic syndromes, including minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN) (also called membranous glomerulonephritis, MGN), and membranoproliferative glomerulonephritis (MPGN). For years pathologists found no changes in MCD tissue when viewing specimens under light microscopy, hence the name minimal change disease. With the advent of electron microscopy, the changes now known as the hallmarks for the disease include diffuse loss of podocyte foot processes, vacuolation of the podocyte foot processes, and growth of microvilli on the visceral epithelial cells. Diabetic nephropathy is the most common cause of nephrotic syndrome in developed countries.

Hypertriglyceridemia may occur due to changes in the activity of enzymes that degrade triglycerides, such as lipoprotein lipase (2-4).

The molecular basis of nephrotic syndrome is not known. Furthermore, the association of proteinuria and glucocorticoid sensitivity in nephrotic syndrome and the link between proteinuria and hypertriglyceridemia, two key components of nephrotic syndrome, have yet to be established. Therapy designed to reduce proteinuria further complicates the study of disease mechanisms. For example, glucocorticoids used to treat proteinuria in MCD independently raise plasma triglyceride levels (5), and normalization of plasma triglyceride levels lags behind the response of proteinuria to glucocorticoids in certain forms of nephrotic syndrome, such as MCD (6).

The present disclosure provides a disclosure of the biochemical basis of nephrotic syndrome (exemplified by a model of MCD) and provides an explanation for the observed proteinuria and other effects. As a result, the present disclosure provides method for treating and/or preventing nephrotic syndrome, such as but not limited to, diabetic nephropathy, MCD, FSGS, MN/MGN, and MPGN, as well as methods of alleviating symptoms associated with nephrotic syndrome, including, but not limited to, proteinuria, hypercholesterolemia, hypertriglyceridemia, hypoalbuminemia and edema. The present disclosure further provides methods for treating and preventing diabetic conditions and physiological effects thereof.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1I shows Angptl4 mRNA and protein expression in experimental and human MCD.

FIG. 1A shows induction of nephrotoxic serum (NTS) induced heterologous phase proteinuria (n = 4 rats/group); *** P<0.001.

FIG. 1B shows glomerular Angptl4 mRNA expression was upregulated during nephrotoxic serum (NTS) induced heterologous phase proteinuria shown in FIG. 1A (n = 4 rats/group); *** P<0.001.

FIG. 1C shows NTS injected Angptl4 -/- mice (n = 4 mice/group) develop significantly lower albuminuria, indicating a role of Angptl4 in the disease process; * P<0.05.

FIG. 1D shows NTS injected Angptl4 -/- mice (n = 4 mice/group) developed only 30% foot process effacement compared to diffuse effacement in Angptl4 +/+ mice.

FIG. 1E shows by confocal imaging that Angptl4 in normal rat glomeruli co-localized with podocyte protein CD2AP, indicating expression in podocytes. Absorbing out reactivity from anti-Angptl4 antibody with recombinant Angptl4 abolished immunoreactivity.

FIG. 1F shows significant glomerular mRNA upregulation of Angptl4 was seen after injection of a single dose of puromycin aminonucleoside (PAN model) starting on Day 3 (peak up to 80 fold increase in different studies). Lesser upregulation developed in PHN, and no significant change was detected in anti-Thy1.1 nephritis or a rat model of non-HIV collapsing focal and segmental glomerulosclerosis (CG) using sieved glomeruli or glomeruli isolated by laser capture microdissection (LCM) [threshold for significance is 3-fold change (7)].

FIG. 1G shows glomeruli had increased Angptl4 expression (red) that overlapped partially (white arrows) with GBM heparan sulfate proteoglycans (white, intensity reduced in PAN) and podocyte nephrin (green) on day 6 after injection of a single dose of puromycin aminonucleoside (PAN model).

FIG. 1H shows Immunogold EM of PAN Model day 6 kidney demonstrating gold particles in the podocyte (yellow arrows) and GBM (black arrows); magnification: EM 40,000x.

FIG. 1I shows kidney biopsies from MCD patients (n = 5 patients) revealed increased glomerular expression of Angptl4 (red) that overlaps substantially with nephrin and GBM laminin, and only marginally with endothelial PECAM1 staining; magnification: light microscopy 400x.

FIG. 2 shows the generation and characterization of male Angptl4 transgenic rats.

FIG. 2A show a transgenic (TG) rat model for podocyte specific over expression of Angptl4 in podocytes.

FIG. 2B shows tissue specific over expression of Angptl4 mRNA (n = 3 rats/group) consistent with expression in podocytes; *** P < 0.001.

FIG. 2C shows PAS stained sections from 3 month old heterozygous TG rats (n = 3 rats/group) and revealed normal morphology (magnification 400x), with prominent podocytes in NPHS2-Angptl4 TG rats (arrows).

FIG. 2D shows confocal imaging and revealed increased glomerular expression of Angptl4 protein (magnification 400x) that overlapped with podocyte nephrin (magnification 630x) and GBM laminin (magnification 630x) in NPHS2-Angptl4 TG rats (n = 3 rats/group).

FIG. 2E shows that by age 5 months, diffuse foot process effacement was noted on EM in homozygous NPHS2-Angptl4 TG rats.

FIG. 2F shows immunogold EM analysis of these rats and revealed a progression from intact foot processes (FP) containing gold particle clusters and scattered GBM particles at age 1 month, to partial effacement with GBM gold particle clusters opposite to effaced foot processes (EFP) reaching up to the endothelial (ENDO) surface, and finally diffuse effacement with dense gold particle clusters in the GBM by age 5 months. electron microscopy magnification 15,000x – 40,000x.

FIG. 3 shows the relationship of Angptl4 overexpression with proteinuria.

FIG. 3A shows female homozygous NPHS2-Angptl4 TG rats developed significant albuminuria (n = 6 rats/group); * P<0.05; ** P < 0.01, *** P<0.001.

FIG. 3B shows male heterozygous NPHS2-Angptl4 TG rats developed significant albuminuria (n = 6 rats/group); * P<0.05; ** P < 0.01, *** P<0.001.

FIG. 3C shows male homozygous NPHS2-Angptl4 TG rats developed significant albuminuria (n = 6 rats/group); ** P < 0.01.

FIG. 3D shows GelCode blue stained SDS PAGE assessment of urinary protein (3 μg / lane, except MCD remission) followed by densitometry of the bands (n = 3 readings / value) and shows a predominance of albuminuria (intact albumin band at 70 kDa, arrow) in NPHS2-Angptl4 TG rats similar to human MCD.

FIG. 3E shows immunogold EM with anti-V5 antibody to specifically detect transgenic protein in 3 month heterozygous TG male rats and showed gold particles in effaced foot processes (EFP) and GBM in NPHS2-Angptl4 TG rats; EM 40,000x.

FIG. 3F shows induction of PAN in heterozygous male NPHS2-Angptl4 (low dose) worsened, proteinuria compared to wild type littermates (n = 8 rats/group); * P<0.05; ** P < 0.01, *** P<0.001.

FIG. 3G shows rats treated with glucocorticoids (PAN-S) on alternate days starting 1 day after induction of PAN showed transient reduction in Day 6 proteinuria (n = 4 rats/group); * P<0.05; ** P < 0.01,

FIG. 3H is a study of glomerular gene expression from the study in 3G, and shows that Angptl4 is a glucocorticoid sensitive gene. * P<0.05; ** P < 0.01

FIG. 4 shows the relationship of Angptl4 sialylation to proteinuria.

FIG. 4A shows 2D gel electrophoresis (200 μg protein / gel) and Western blot of protein from perfused glomeruli (upper panel, control; middle panel PAN; lower panel PAN plus the addition of glucocorticoid). In the control panel, analysis revealed the presence of small amounts of fragments (red arrow; 1) and monomers (yellow arrow; 2) of Angptl4, and larger amounts of low order oligomers (pink arrows; 3) of Angptl4 migrating at neutral or high pI. On PAN Day 6 (middle panel), high and neutral pI oligomeric forms were increased (pink arrow, 3; orange arrow 4), whereas treatment with glucocorticoids (see FIG. 3G) blunts this increase (lower panel). Of the Angptl4 migrating at neutral pI, fragments were reactive with anti - phosphothreonine antibodies, whereas oligomers are reactive with sialic acid binding lectin MAA (exemplified for PAN, excerpts from independent blots).

FIG. 4B shows densitometry analysis of the Western blot from FIG. 4A; ** P<0.01; *** P<0.001.

FIG. 4C shows 2D gel electrophoresis (150 μg protein / gel) and Western blot of proteins from Angptl4-HEK293 stable cell line incubated with control and probed with antibodies to MAA lecithin (top panel), anti Angplt4 antibodies (upper middle panel); Angptl4-HEK293 stable cell line incubated with sialic acid precursor ManNAc (25 mM) and probed with anti-Angptl4 antibodies (lower middle panel) and Angptl4-HEK293 stable cell line incubated with sialic acid precursor ManNAc (25 mM) and probed with antibodies to MAA lecithin (lower panel). Analysis of the concentrated supernatant revealed a shift from high pI forms (green arrow and line in upper middle panel) towards neutral pI forms (blue arrow in lower middle panel and lower panel), that were MAA reactive.

FIG. 4D shows that feeding NPHS2-Angptl4 TG rats with 1 mg/ml ManNAc in tap water for 12 days (d) caused a significant reduction in 18 hour albuminuria (nadir on

Day 12 was 59.4 ± 3.3% of baseline), which returned towards baseline values after 12 days of washout, and approached untreated control NPHS2-Angptl4 TG rat values on day 24 of washout (individual tracings and pilot study data in FIG. 6). In panel d, all * differences are with baseline values. * P<0.05; ** P<0.01; *** P<0.001.

FIG. 5 shows characterization of recombinant Angptl4 produced by a HEK293 stable cell line.

FIG. 5A shows HEK293 cells stably transfected with a pcDNA-rat Angptl4-V5-His expression construct line showed 55,000 fold upregulation of Angptl4 mRNA expression compared to a control empty vector cell line. *** P < 0.001

FIG. 5B shows Angptl4-HEK293 cells secreted mostly intact protein into the supernatant under serum free conditions (as demonstrated by 2D gel electrophoresis and Western blot with pre-immune serum and anti-Angptl4 antibodies).

FIG. 5C shows 2D gel electrophoresis of 200 ug human plasma (n=4 patients/group) and demonstrates that only in patients with MCD relapse was elevated circulating levels of the 55-70 kDa pI 8-8.5 Angptl4 protein observed (oval in MCD relapse panel). Increased circulating levels of neutral PI monomers and oligomers were observed in MCD patients in relapse (arrow in in MCD relapse panel) and monomers only in patients with MN (arrow in MN panel).

FIG. 6 shows administration of ManNAc reduced albuminuria in a rat model.

FIG. 6A shows representative tracing of albuminuria from pilot studies with heavily albuminuric NPHS2-Angptl4 TG rats receiving increasing doses of ManNAc (1 to 8 mg/ml). The study revealed that ManNAc reduced albuminuria in these animals.

FIG. 6B shows individual tracings from the study group of NPHS2-Angptl4 TG rats treated with ManNAc (1 mg/ml) that completed the study. Two urine collections 12 days apart were conducted prior to the start of the study to ensure that these rats developed increasing albuminuria with time. The study revealed that ManNAc (1 mg/ml) reduced albuminuria in these animals.

FIG. 6C shows 2D electrophoresis and Western blotting (200 μg protein / gel) of glomeruli from Day 12 ManNAc or control TG rats using anti-Angptl4 antibodies or lectin SNA I (n = 3 blots / condition). Reproducible neutral pI Angptl4 spots are bordered by red oval (1), and high pI by green oval (2). Neutral pI spots are also reactive with sialic acid binding lectin SNA I.

FIG. 6D shows densitometry of Angptl4 spots of FIG. 6C and indicates a significant increase in the percentage of neutral pI forms in ManNAC fed rats. *** P<0.001.

FIG. 7 shows improvement in nephrotic syndrome parameters in ManNAc treated rats with puromycin aminonucleoside nephrosis (PAN model). In FIGS. 7A-7D, rats (n = 5 rats/group) received a single intravenous injection of puromycin aminonucleoside (10 mg/100 gm), and were treated with ManNAC 80 mg/Kg body weight in tap water starting on Day 4, which coincides with the onset of proteinuria.

FIG. 7A shows a significant reduction in proteinuria in ManNAc treated rats on day 6 post administration of puromycin aminonucleoside. * P<0.05; ** P<0.01; *** P<0.001.

FIG. 7B shows a significant improvement in plasma albumin levels in ManNAc treated rats on day 6 post administration of puromycin aminonucleoside. * P<0.05; ** P<0.01; *** P<0.001.

FIG. 7C shows a significant reduction in hypercholesterolemia in ManNAc treated rats on day 6 post administration of puromycin aminonucleoside. * P<0.05; ** P<0.01; *** P<0.001.

FIG. 7D shows a significant reduction in hypertriglyceridemia in ManNAc treated rats on day 6 post administration of puromycin aminonucleoside. * P<0.05; ** P<0.01; *** P<0.001.

FIG. 8 shows the effect of ManNAc therapy on proteinuria in diabetic animals.

FIG. 8A shows 2D gel electrophoresis and Western blot analysis of glomeruli from diabetic *db/db* and control *db/m* mice using an anti-Angptl4 Ab. The results show increased expression of Angptl4 in diabetic mouse glomeruli as compared to control, including the high pI forms that are involved in the pathogenesis of proteinuria.

FIG. 8B shows 2D gel electrophoresis and Western blot analysis of glomeruli from Zucker Diabetic Fatty rats and control normoglycemic rats using an anti-Angptl4 Ab. The results show increased expression of Angptl4 in rat glomeruli from Zucker Diabetic Fatty rats as compared to control, including the high pI forms that are involved in the pathogenesis of proteinuria.

FIG. 8C shows ManNac treatment decreased proteinuria in Zucker Diabetic Fatty rats. 13 week old male Zucker Diabetic Fatty rats were treated with ManNAc in tap water or with plain tap water (n = 4 rats/group) at 70 and 140 mg/Kg. Significant reduction in proteinuria was noted in the ManNAc treated group. * P<0.05; ** P<0.01.

FIG. 9 shows the amino acid and cDNA sequences of Angptl4 from various species. SEQ ID NOS. 1 and 2 show amino acid and cDNA sequence from human (Protein Variant 1 isoform a, long form; underlined amino acid sequences at a position 40 and

161-164); SEQ ID NOS. 3 and 4 show amino acid and cDNA sequence from human (Protein Variant 3 isoform b, short form; underlined amino acid sequences at a position 40 and 161-164); SEQ ID NOS. 5 and 6 show amino acid and cDNA sequence from rat; SEQ ID NOS: 7 and 8 show amino acid and cDNA from mouse.

SUMMARY OF THE DISCLOSURE

In a first aspect, the present disclosure provides methods of treatment and/or prevention of nephrotic syndrome in a subject. In one embodiment, the nephrotic syndrome is characterized as minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN) / membranous glomerulonephritis (MGN) membranoproliferative glomerulonephritis (MPGN), and diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of nephrotic syndrome, thereby treating or preventing nephrotic syndrome in the subject.

In a second aspect, the present disclosure provides methods of treatment and/or prevention of MCD in a subject. The methods comprise the step of administering to a subject a sialic acid or sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of nephrotic syndrome, thereby treating or preventing nephrotic syndrome in the subject.

In a third aspect, the present disclosure provides methods of alleviating one or more symptoms of nephrotic syndrome, such as, but not limited to, proteinuria, hypercholesterolemia, hypertriglyceridemia and edema. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition

or in combination with a secondary agent. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of nephrotic syndrome, thereby alleviating one or more symptoms of nephrotic syndrome in the subject.

In a fourth aspect, the present disclosure provides methods for reducing proteinuria in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In another embodiment, the subject is suffering from a diabetic condition, such as, but not limited to, diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the proteinuria is caused by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the induction of proteinuria thereby reducing proteinuria in the subject.

In a fifth aspect, the present disclosure provides methods of reducing edema in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In a specific embodiment, the edema is caused by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of the polypeptide, such as, but not limited to Angptl4, involved in the induction of edema thereby reducing edema in the subject.

In a sixth aspect, the present disclosure provides methods of reducing hypercholesterolemia in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as

MCD, FSGS, MN /MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In a specific embodiment, the hypercholesterolemia is caused, at least in part, by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of the polypeptide, such as, but not limited to Angptl4, involved in the induction of hypercholesterolemia thereby reducing hypercholesterolemia in the subject.

In a seventh aspect, the present disclosure provides methods of reducing hypertriglyceridemia in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In a specific embodiment, the hypertriglyceridemia is caused by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of the polypeptide, such as, but not limited to Angptl4, involved in the induction of hypertriglyceridemia thereby reducing hypertriglyceridemia in the subject.

In an eight aspect, the present disclosure provides methods of treatment and/or prevention of diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the foregoing conditions are caused by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. The methods comprise the step of administering to a subject sialic acid or a sialic acid precursor. The sialic acid or sialic acid precursor may be administered at a therapeutically effective dose, either alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such administration restores normal sialylation of the polypeptide, such as, but not limited to Angptl4, thereby treating or preventing the foregoing conditions in the subject.

In a ninth aspect, the present disclosure provides a composition for use in the methods of the first through eight aspects. The composition comprises sialic acid or one or more sialic acid precursors. In one embodiment, the sialic acid precursor is ManNAc. In an alternate embodiment, the sialic acid precursor is a derivative of ManNAc. Such composition may contain ManNAc and one or more derivatives of ManNAc as well as a secondary agent.

In a tenth aspect, the present disclosure provides for methods of determining the status of a subject with respect to nephrotic syndrome or a diabetic condition. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy. In one embodiment, such methods determine in the subject the level of a polypeptide associated with nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, the level of sialylation of a polypeptide associated with nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, or a combination of the foregoing. The amount and/or level of sialylation of the polypeptide as determined from the subject may be compared to corresponding amounts and levels from a subject that is diagnosed as not suffering from nephrotic syndrome or a diabetic condition (control subject). Such amounts and levels may also be compared to a reference standard. A decrease in the level of sialylation as compared to the control subject or reference standard indicates the subject is suffering from or at risk for, nephrotic syndrome or a diabetic condition; the level of sialylation may be determined with respect to the high pI form of the polypeptide (which is hyposialylated).

In an eleventh aspect, the present disclosure provides for methods of determining the efficacy of a treatment for nephrotic syndrome or a diabetic condition in a subject undergoing treatment for nephrotic syndrome or a diabetic condition. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy. The amount

and/or level of sialylation of the polypeptide as determined from the subject during treatment may be compared to corresponding amounts and/or levels from the subject prior to initiating treatment. An increase in the level of sialylation in the subject undergoing treatment as compared to the level of sialylation determined prior to initiating treatment indicates the treatment is having the desired effect; the level of sialylation may be determined with respect to the high pI form of the polypeptide (which is hyposialylated). Furthermore, the amount and/or level of the polypeptide as determined from the subject during treatment may be compared to corresponding amounts and/or levels a subject that is diagnosed as not suffering from nephrotic syndrome or a diabetic condition (control subject). Such amounts and levels may also be compared to a reference standard. A level of sialylation obtained from the subject during treatment that is equal to or approaching the level of sialylation from the control subject or reference indicates the treatment is having the desired effect; the level of sialylation may be determined with respect to the high pI form of the polypeptide (which is hyposialylated).

In a twelfth aspect, the present disclosure provides for methods of identifying a compound effective for treating or preventing nephrotic syndrome, a diabetic condition or a condition associated therewith. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy. In general, such screening methods comprises the steps of providing an assay system (as described in more detail below) that expresses a polypeptide involved in the etiology of nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, introducing into the assay system a test compound to be tested and determining whether the effect of the test compound on the level of sialylation of the polypeptide.

DETAILED DESCRIPTION

In the following discussion certain articles and methods will be described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate,

where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

While investigating the pathogenesis of nephrotic syndrome and proteinuria associated therewith, γ2-nephtotoxic serum (NTS, a serum raised against whole glomeruli), was injected into rats and a panel of differentially expressed glomerular genes was identified (7). Two genes not previously known to be involved in the pathogenesis of nephrotic syndrome and proteinuria were identified. One of these genes encoded for the transcriptional factor zinc fingers and homeoboxes 3 (ZHX3) expressed in podocytes, and has now been shown to play a key role in the pathogenesis of MCD (7). The second gene, angiopoietin-like 4, *ANGPTL4*, was highly upregulated in the podocyte. The expression of the *ANGPTL4* gene was analyzed in animal models of human glomerular disease, including puromycin nephrosis (PAN), a model of MCD, passive Heymann nephritis (PHN), a model of membranous nephropathy (MN), and anti-Thy1.1 nephritis, a model of mesangial injury.

Angiopoietin-like proteins have been implicated in the development of hypertriglyceridemia and tumor metastasis, and are functionally distinct from the angiopoietins. Angptl4 is a PPARγ (8) and PPARα (9) target gene highly expressed in the liver and adipose tissue, strongly induced by fasting in white adipose tissue and liver, and is an apoptosis survival factor for vascular endothelial cells under normoxic conditions (10). Angptl4 is a potent inhibitor of LPL (11), inducing significant hypertriglyceridemia following intravenous injection or adenovirus-mediated expression (12, 13). Other studies showed lesser expression of Angptl4 in cardiomyocytes and skeletal muscle, and low level expression in whole kidney on Northern blot analysis (8). Recent population based studies of the *ANGPTL4* gene reveals variants that affect triglyceride levels in humans (14, 15).

A role of Angptl4 in proteinuria has not been previously reported. The present disclosure shows a conclusive role of podocyte secreted Angptl4 in the etiology of in nephrotic syndrome and diabetic conditions. The present disclosure demonstrates for the first time that a large part of podocyte secreted Angptl4 in experimental one form of nephrotic syndrome is hyposialylated and that improving sialylation dramatically reduces proteinuria, edema, hypercholesterolemia and hypertriglyceridemia and normalizes electrophoretic migration of Angptl4. Angptl4 is the first glucocorticoid

sensitive gene to be directly implicated in the pathogenesis of nephrotic syndrome and also the first direct link between proteinuria and hypertriglyceridemia in nephrotic syndrome. Angptl4 amino acid and cDNA sequences from human (Protein Variant 1 isoform a, long form and Protein Variant 3 isoform b, short form), rat and mouse are shown in FIG. 8.

Definitions

The terms "prevention", "prevent", "preventing", "suppression", "suppress" and "suppressing" as used herein refer to a course of action (such as administering a compound or pharmaceutical composition) initiated prior to the onset of a symptom, aspect, or characteristics of a disease or condition so as to prevent or reduce such symptom, aspect, or characteristics. Such preventing and suppressing need not be absolute to be useful.

The terms "treatment", "treat" and "treating" as used herein refers a course of action (such as administering a compound or pharmaceutical composition) initiated after the onset of a symptom, aspect, or characteristics of a disease or condition so as to eliminate or reduce such symptom, aspect, or characteristics. Such treating need not be absolute to be useful.

The term "in need of treatment" as used herein refers to a judgment made by a caregiver that a patient requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the patient is ill, or will be ill, as the result of a disease or condition that is treatable by a method or compound of the disclosure.

The term "in need of prevention" as used herein refers to a judgment made by a caregiver that a patient requires or will benefit from prevention. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the patient will be ill or may become ill, as the result of a disease or condition that is preventable by a method or compound of the disclosure.

The term "individual", "subject" or "patient" as used herein refers to any animal, including mammals, such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and humans. The term may specify male or female or both, or exclude male or female.

The term "therapeutically effective amount" as used herein refers to an amount of a compound, either alone or as a part of a pharmaceutical composition, that

is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease or condition. Such effect need not be absolute to be beneficial. When referring to sialic acid or a sialic acid precursor, the term "therapeutically effective amount" refers to an amount of sialic acid or the sialic acid precursor sufficient to restore normal sialylation patterns to a polypeptide, such as, but not limited to, Angltl4 or an amount of sialic acid or the sialic acid precursor sufficient to reduce proteinuria or another symptom of nephrotic syndrome in a subject.

The term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, salt of an ester, solvate or other derivative of sialic acid or a sialic acid precursor of the present disclosure that, upon administration to a subject, is capable of providing (directly or indirectly) sialic acid or a sialic acid precursor of the disclosure or a metabolite or residue thereof. Particularly favored derivatives are those that increase the bioavailability of sialic acid or the sialic acid precursor of the disclosure when such are administered to a subject (e.g., by allowing an orally administered compound to be more readily absorbed into the blood), enhance delivery of the sialic acid precursor to a given biological compartment, increase solubility to allow administration by injection, alter metabolism or alter rate of excretion. In one embodiment, the derivative is a prodrug.

The term "pharmaceutically acceptable salt(s)", unless otherwise indicated, includes salts of acidic or basic groups that may be present in sialic acid or the sialic acid precursor of the present disclosure.

The terms "about" and "approximately" shall generally mean an acceptable degree of error or variation for the quantity measured given the nature or precision of the measurements. Typical, exemplary degrees of error or variation are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. For biological systems, the term "about" refers to an acceptable standard deviation of error, preferably not more than 2-fold of a give value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

Methods of Treatment and Prevention

The present disclosure provides methods of treatment and/or prevention of nephrotic syndrome. The present disclosure further provides methods of treatment and/or prevention of MCD. The present disclosure additionally provides methods of

alleviating one or more symptoms of nephritic syndrome, such as, but not limited to, proteinuria, hypercholesterolemia, hypertriglyceridemia and edema. Still further, the present disclosure provides methods for reducing proteinuria. Further still, the present disclosure provides methods for reducing edema. The present disclosure also provides methods for reducing hypercholesterolemia and hypertriglyceridemia The present disclosure also provides methods for the treatment and/or prevention of a diabetic condition or a physiological condition associated therewith. The present disclosure additionally provides for pharmaceutical compositions comprising sialic acid or one or more sialic acid precursors or combinations of the foregoing.

In one embodiment, the teachings of the present disclosure provide for the treatment and/or prevention of nephrotic syndrome in a subject in need of such treatment or prevention. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of nephrotic syndrome. Such administration thereby treats and/or prevents nephrotic syndrome in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such treatment and/or prevention.

In an alternate embodiment, the teachings of the present disclosure provide for the treatment and/or prevention of MCD in a subject in need of such treatment or prevention. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of MCD. Such administration thereby treats and/or prevents MCD in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such treatment and/or prevention.

In further embodiment, the teachings of the present disclosure provide for methods of alleviating one or more symptoms of nephrotic syndrome, such as, but not limited to, proteinuria, hypercholesterolemia, hypertriglyceridemia and edema. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephroapthy. In another embodiment, the nephrotic syndrome is characterized as MCD. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of nephrotic syndrome. Such administration thereby alleviates one or more symptoms of nephrotic syndrome, such as, but not limited to, proteinuria and edema, nephrotic syndrome in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such treatment and/or prevention.

In still a further embodiment, the teachings of the present disclosure provide methods for reducing proteinuria in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephroapthy. In another embodiment, the nephrotic syndrome is characterized as MCD. In another embodiment, the subject is suffering from a disorder characterized by proteinuria, such as, but not limited to, diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the proteinuria is caused, at least in part, by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of proteinuria. Such administration thereby reduces proteinuria in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such reduction.

In yet a further embodiment, the teachings of the present disclosure provide methods for reducing edema in a subject. In one embodiment, the subject is suffering

from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In a specific embodiment, the edema is caused, at least in part, by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. Such method comprises the step of administering sialic acid or a sialic acid precursor or a combination of the foregoing to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of proteinuria. Such administration thereby reduces proteinuria in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such reduction.

In yet a further embodiment, the teachings of the present disclosure provide methods for reducing hypercholesterolemia in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In a specific embodiment, the hypercholesterolemia is caused, at least in part, by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of hypercholesterolemia. Such administration thereby reduces hypercholesterolemia in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such reduction.

In yet a further embodiment, the teachings of the present disclosure provide methods for reducing hypertriglyceridemia in a subject. In one embodiment, the subject is suffering from nephrotic syndrome. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In a specific embodiment, the hypertriglyceridemia is caused, at least in part,

by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of hypertriglyceridemia. Such administration thereby reduces hypertriglyceridemia in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such reduction.

In yet a further embodiment, the teachings of the present disclosure provide methods for treatment and/or prevention of a diabetic condition in a subject or a physiological condition associated therewith. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy. In one embodiment, the physiological condition associated with the diabetic condition is proteinuria. In a specific embodiment, the diabetic condition is caused, at least in part, by hyposialylation of a polypeptide, such as, but not limited to, Angptl4. Such method comprises the step of administering sialic acid or a sialic acid precursor to the subject. Such administration restores normal sialylation of a polypeptide, such as, but not limited to Angptl4, involved in the etiology of the diabetic condition. Such administration thereby treats and/or prevents the diabetic condition in the subject. The sialic acid or sialic acid precursor may be administered at a therapeutically effective amount. Furthermore, sialic acid or the sialic acid precursor may be administered alone, as a part of a pharmaceutical composition or in combination with a secondary agent. Such method may further comprise identifying a subject in need of such treatment and/or prevention.

Methods of Diagnosis

The present disclosure also provides methods for determining the status of a subject with respect to nephrotic syndrome or a diabetic condition. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy.

In one embodiment, such methods determine in the subject the level of a polypeptide associated with nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, the level of sialylation of a polypeptide associated with nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, or a combination of the foregoing. In a particular embodiment, the level of the high pI form of the polypeptide is determined. This form has been shown to be hyposialylated and indicative of nephrotic syndrome or a diabetic condition. The amount and/or level of sialylation of the polypeptide as determined from the subject may be compared to corresponding amounts and levels from a subject that is diagnosed as not suffering from nephrotic syndrome or a diabetic condition (control subject). Such amounts and levels may also be compared to a reference standard. A decrease in the level of sialylation as compared to the control subject or reference standard indicates the subject is suffering from or at risk for, nephrotic syndrome or a diabetic condition; as discussed above, the level of sialylation may be determined with respect to the high pI form of the polypeptide (which is hyposialylated).

The present disclosure further provides methods for determining the efficacy of a treatment for nephrotic syndrome or a diabetic condition in a subject undergoing treatment for nephrotic syndrome or a diabetic condition. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy. In another embodiment, the nephrotic syndrome is characterized as MCD. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy.

In one embodiment, such methods determine in the subject the level of a polypeptide associated with nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, the level of sialylation of a polypeptide associated with nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, or a combination of the foregoing. In a particular embodiment, the level of the high pI form of the polypeptide is determined. This form has been shown to be hyposialylated and indicative of nephrotic syndrome or a diabetic condition. The amount and/or level of sialylation of the polypeptide as determined from the subject during treatment may be compared to corresponding amounts and/or levels from the subject prior to initiating treatment. An increase in the level of sialylation in the subject undergoing treatment as compared to the level of sialylation determined prior to initiating

treatment indicates the treatment is having the desired effect; as discussed above, the level of sialylation may be determined with respect to the high pI form of the polypeptide (which is hyposialylated). Furthermore, the amount and/or level of the polypeptide as determined from the subject during treatment may be compared to corresponding amounts and/or levels a subject that is diagnosed as not suffering from nephrotic syndrome or a diabetic condition (control subject). Such amounts and levels may also be compared to a reference standard. A level of sialylation obtained from the subject during treatment that is equal to or approaching the level of sialylation from the control subject or reference indicates the treatment is having the desired effect; as discussed above, the level of sialylation may be determined with respect to the high pI form of the polypeptide (which is hyposialylated).

Any method known in the art for determining protein levels and levels of sialylation may be used in such methods, including, but not limited to, any methods described herein.

FIG. 5C shows the utility of this approach. 200 μg human plasma from patients (n = 4 patients/group) with various conditions were analyzed by 2D gel electrophoresis and Western blots were prepared using anti-Angptl4 antibodies. This figure demonstrates that only patients with MCD relapse had circulating hyposialylated form of Angptl4 polypeptide (this 55 - 70 kDa pI 8 – 8.5 form of the Angptl4 polypeptide is indicated by the oval); this polypeptide was absent in patients with MCD remission. Increased circulating neutral pI monomers and oligomers were noted in MCD patients in relapse (arrow), and monomers only in MN (arrow).

Any of the above methods of treatment may be used in conjunction with the methods of administering a polypeptide described in US provisional Application Nos. 61/351,866 and 61/483,854.

Methods of Screening

The present disclosure also relates to a method for identifying a compound effective for treating or preventing nephrotic syndrome, a diabetic condition or a condition associated therewith, such as, but not limited to, proteinuria, hypercholesterolemia, hypertriglyceridemia or edema. In one embodiment, the nephrotic syndrome is characterized as MCD, FSGS, MN or MGN. In another embodiment, the nephrotic syndrome is characterized as MCD. In one embodiment, the diabetic condition is diabetic nephropathy, diabetes mellitus, lupus nephritis or

primary glomular disease. In a specific embodiment, the diabetic condition is diabetic nephropathy. Such compounds may be useful as active ingredients included in pharmaceutical compositions or for administration alone. In one embodiment, the methods include determining the level of sialylation of a polypeptide involved in the etiology of nephrotic syndrome, such as, but not limited to, Angptl4.

In general, such screening methods comprises the steps of providing an assay system (as described in more detail below) that expresses a polypeptide involved in the etiology of nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, introducing into the assay system a test compound to be tested and determining whether the effect of the test compound on the level of sialylation of the polypeptide. The methods involve the identification of candidate or test compounds or agents (polypeptides, functional nucleic acids, carbohydrates, antibodies, small molecules or other molecules) which effect the level of sialylation of the polypeptide. Such compounds may then be further tested in appropriate systems (such as, but not limited to, the animal models systems described herein) to determine the activity of the identified compounds.

Candidate compounds are identified using a variety of assays, such as, but not limited to, assays that employ cells which express a polypeptide involved in the etiology of nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4 or in assays with isolated polypeptides. The various assays can employ a variety of variants of such polypeptides (e. g., full-length, a biologically active fragment, or a fusion protein which includes all or a portion of the desired polypeptide). Moreover, such polypeptides can be derived from any suitable mammalian species (e. g., human, rat or murine); in a specific embodiment, the polypeptide is derived from a human.

Where the assay involves the use of a whole cell, the cell may either naturally express a polypeptide involved in the etiology of nephrotic syndrome or a diabetic condition, such as, but not limited to, Angptl4, or may be modified to express the same. In the latter case, cells can be modified to express a desired polypeptide through conventional molecular biology techniques, such as by infecting the cell with a virus comprising such polypeptide. The cell can also be a prokaryotic or an eukaryotic cell that has been transfected with a nucleotide sequence encoding such polypeptide. In the foregoing, full length polypeptides, fragments or fusion proteins

containing at least a part of such polypeptide may be used. Exemplary assay systems are described in the current specification.

The various screening assays may be combined with an *in vivo* assay entailing measuring the effect of the test compound on the symptoms the disease states and conditions discussed herein. In such an embodiment, the compounds may be evaluated to determine if they impact a parameter associated with nephrotic syndrome or a diabetic condition or a condition related thereto, such as, but not limited to, proteinuria hypercholesterolemia, hypertriglyceridemia or edema. Such parameters include, but are not limited to, determining 1) the level of sialylation of a polypeptide involved in the etiology of hypercholesterolemia, reduces hypertriglyceridemia or related conditions, such as, but not limited to Angptl4 and 2) the amount of high pI forms a polypeptide involved in the etiology of hypercholesterolemia, reduces hypertriglyceridemia or related conditions, such as, but not limited to Angptl4 (these forms are hyposialylated); 3) determining the level of protein excretion, either total or with regard to specific components; and 4) determining the impact of the test compound on kidney morphology, such as, but not limited, the podocyte.

In one embodiment, such a screening assay can be performed, for example, by determining the level of sialylation of a polypeptide involved in the etiology of hypercholesterolemia, reduces hypertriglyceridemia, such as, but not limited to, Angptl4 and detecting a difference in the level of sialylation of such polypeptide in the presence of as compared to the absence of a test compound. In a particular embodiment, the high pI forms of such polypeptide are specifically examined (this form is hyposialylated). Such screening assay may be in vitro, in vivo or ex vivo and may be cell culture based (either with whole cells or lysates) or may be based on an animal model. Any assay of the present disclosure may be used in the foregoing method.

Suitable test compounds for use in the screening methods can be obtained from any suitable source, such as conventional compound libraries. The test compounds can also be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are

applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. Examples of methods for the synthesis of molecular libraries can be found in the art. Libraries of compounds may be presented in solution or on beads, bacteria, spores, plasmids or phage.

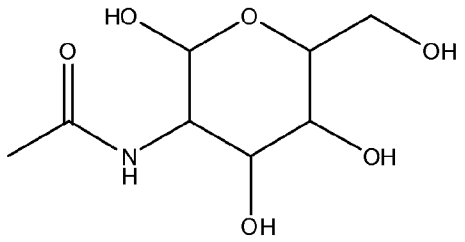
In the foregoing methods, the high pI forms of Angptl4 refer to a polypeptide migrating at 55 - 70 kDa and having a pI of 8 – 8.5.

The present disclosure also provides kits for carrying out any method of the present disclosure, which can contain any of the compounds and/or compositions disclosed herein or otherwise useful for practicing a method of the disclosure.

Sialic Acid Precursors

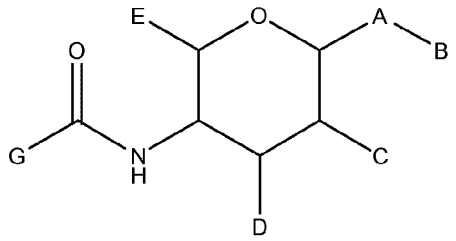
The present disclosure provides for various uses of sialic acid (N-acetylneuraminic acid) or a sialic acid precursor.

In one embodiment, the sialic acid precursor is N-acetylmannosamine (ManNAc; also referred to as 2-Acetamido-2-deoxy-D-mannose or N-acetyl-D-mannosamine). The structure of ManNAc is shown in formula I below.



I

In an alternate embodiment, the sialic acid precursor is defined by the formula Ia below.



Ia

Wherein:

A is CH_2 or NH;

B, C, D and E are each independently selected from the group consisting of: H, OH, X, O-CO-X or O-X, wherein X is a substituted or unsubstituted alkyl or alkenyl, X (when present) being selected independently for each group B, C,D and E;

G is H, OH, Y or O-Y, wherein Y is a substituted or unsubstituted alkyl or alkenyl.

It is understood that the compounds of Formula I and Ia can also be represented in the chair configuration as well.

In one embodiment, X and/or Y are independently a C1 to C5 alkyl. In a particular embodiment, X and/or Y are propyl; in a further embodiment, X and/or Y are butyl.

In one embodiment, at least one of B, C, D, E or G is not H or OH. In a further embodiment, at least two of B, C, D, E or G is not H or OH. In still a further embodiment, at least three of B, C, D, E or G is not H or OH. In yet another embodiment, none of B, C, D, E or G is not H or OH.

In a particular embodiment, G is CH_3, A is CH_2, and at least one of B, C, D or E is not H or OH. In a further particular embodiment, G is CH_3, A is CH_2, and at least two of B, C, D or E are not H or OH. In still a further particular embodiment, G is CH_3, A is CH_2, and at least three of B, C, D or E are not H or OH. In yet a further particular embodiment, G is CH_3, A is CH_2, and all of B, C, D or E are not H or OH. In the foregoing, in one embodiment, B, C and D may each independently be CO-X.

It is noted that where G is CH_3, A is CH_2, and B, C, D and E are each OH, the compound is ManNAc (Formula I).

Representative derivatives falling under the formula I include, but are not limited to, Bu4ManNAc, 3,4,6-O-Bu3ManNAc or 1,3,4-O-Bu3ManNAc (such compounds are described in Aich, et al, Glycoconj J. DOI 10.1007/s10719-010-9292-3 accepted April 14, 2010).

Additional sialic acid precursors include N-levulinoyl sialic acid (SiaLev) and N-levulinoylmannosamine (ManLev) (Charter et al. Glycobiology. 2000 Oct;10(10):1049-56).

All enantiomeric forms of the foregoing compounds are included in the above descriptions.

Compositions

Useful compositions of the present disclosure comprise one or more compounds useful in the treatment and prevention methods of the present disclosure, such as, but not limited to, sialic acid, sialic acid precursors, those compounds identified in the present disclosure or identified by a screening method of the present disclosure. In one embodiment, such compounds increase the sialylation of a polypeptide, such as, but not limited to, Angptl4. In a particular embodiment, the

compounds are sialic acid precursors. Exemplary sialic acid precursors include, but are not limited to, ManNAc and ManNAc derivatives.

The compositions disclosed may comprise one or more of such compounds, in combination with a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington: The Science and Practice of Pharmacy (20th Ed., Lippincott, Williams & Wilkins, Daniel Limmer, editor). To form a pharmaceutically acceptable composition suitable for administration, such compositions will contain an therapeutically effective amount of compound.

The pharmaceutical compositions of the disclosure may be used in the treatment and prevention methods of the present disclosure. Such compositions are administered to a subject in amounts sufficient to deliver a therapeutically effective amount of the compound(s) so as to be effective in the treatment and prevention methods disclosed herein. The therapeutically effective amount may vary according to a variety of factors such as, but not limited to, the subject's condition, weight, sex and age. Other factors include the mode and site of administration. The compositions of the present disclosure may be administered only one time to the subject or more than one time to the subject. Furthermore, when the compositions are administered to the subject more than once, a variety of regimens may be used, such as, but not limited to, one per day, once per week or once per month. The compositions may also be administered to the subject more than one time per day. The therapeutically effective amount and appropriate dosing regimens may be identified by routine testing in order to obtain optimal activity, while minimizing any potential side effects. In addition, co-administration or sequential administration of other agents may be desirable.

The compositions of the present disclosure may be administered in a variety of dosage forms and regimens as discussed herein. Exemplary doses include, but are not limited to, of at least about 0.1 mg/kg to about 750 mg/kg, of at least about 1 mg/kg to about 500 mg/kg, at least about 1 mg/kg to about 200 mg/kg or at least about 1 mg/kg to about 100 mg/kg of body weight. Daily doses of a sailic acid precursor may range from . about 0.1 g/day to about 75 g/day, from about 0.5 g/day to about 50 g/day, from about 1 g/day to about 10 g/day, from about 0.1 g/day to about 5 g/day, from about 0.1 g/day to about 3 g/day, and from about 0.1 g/day to about 1 g/day.

The pharmaceutical compositions may be provided to the subject in any method known in the art. Exemplary routes of administration include, but are not

limited to, oral, subcutaneous, rectal, parenteral, subcutaneous, intramuscular, intraperitoneal, intravenous, topical, epicutaneous, intraosseous, intramuscular, dermal, transdermal, intrathoracic, intrapulmonary, intranasal or pulmonary routes

The compositions of the present disclosure may further comprise agents which improve the solubility, half-life, absorption, etc. of the compound(s). Furthermore, the compositions of the present disclosure may further comprise agents that attenuate undesirable side effects and/or or decrease the toxicity of the compounds(s). Examples of such agents are described in a variety of texts, such a, but not limited to, Remington: The Science and Practice of Pharmacy (20th Ed., Lippincott, Williams & Wilkins, Daniel Limmer, editor).

The compositions of the present disclosure can be administered in a wide variety of dosage forms for administration. For example, the compositions can be administered in forms, such as, but not limited to, tablets, capsules, sachets, lozenges, troches, pills, powders, granules, elixirs, tinctures, solutions, suspensions, elixirs, syrups, ointments, creams, pastes, emulsions, or solutions for intravenous administration or injection. Other dosage forms include administration transdermally, via patch mechanism or ointment. Any of the foregoing may be modified to provide for timed release and/or sustained release formulations.

In the present disclosure, the pharmaceutical compositions may further comprise a pharmaceutically acceptable carriers include, but are not limited to, vehicles, adjuvants, surfactants, suspending agents, emulsifying agents, inert fillers, diluents, excipients, wetting agents, binders, lubricants, buffering agents, disintegrating agents and carriers, as well as accessory agents, such as, but not limited to, coloring agents and flavoring agents (collectively referred to herein as a carrier). Typically, the pharmaceutically acceptable carrier is chemically inert to the active compounds and has no detrimental side effects or toxicity under the conditions of use. The pharmaceutically acceptable carriers can include polymers and polymer matrices. The nature of the pharmaceutically acceptable carrier may differ depending on the particular dosage form employed and other characteristics of the composition.

For instance, for oral administration in solid form, such as but not limited to, tablets, capsules, sachets, lozenges, troches, pills, powders, or granules, the compound(s) may be combined with an oral, non-toxic pharmaceutically acceptable inert carrier, such as, but not limited to, inert fillers, suitable binders, lubricants, disintegrating agents and accessory agents. Suitable binders include, without

limitation, starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include, without limitation, sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthum gum and the like. Tablet forms can include one or more of the following: lactose, sucrose, mannitol, corn starch, potato starch, alginic acid, microcrystalline cellulose, acacia, gelatin, guar gum, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, calcium stearate, zinc stearate, stearic acid as well as the other carriers described herein. Lozenge forms can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acadia, emulsions, and gels containing, in addition to the active ingredient, such carriers as are known in the art.

For oral liquid forms, such as but not limited to, tinctures, solutions, suspensions, elixirs, syrups, the nucleic acid molecules of the present disclosure can be dissolved in diluents, such as water, saline, or alcohols. Furthermore, the oral liquid forms may comprise suitably flavored suspending or dispersing agents such as the synthetic and natural gums, for example, tragacanth, acacia, methylcellulose and the like. Moreover, when desired or necessary, suitable and coloring agents or other accessory agents can also be incorporated into the mixture. Other dispersing agents that may be employed include glycerin and the like.

Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the patient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The compound(s) may be administered in a physiologically acceptable diluent, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol, isopropanol, or hexadecyl alcohol, glycols, such as propylene glycol or polyethylene glycol such as poly(ethyleneglycol) 400, glycerol ketals, such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, an oil, a fatty acid, a fatty acid ester or glyceride, or an acetylated fatty acid glyceride with or

without the addition of a pharmaceutically acceptable surfactant, such as, but not limited to, a soap, an oil or a detergent, suspending agent, such as, but not limited to, pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

Oils, which can be used in parenteral formulations, include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include polyethylene sorbitan fatty acid esters, such as sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol, oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters. Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyldialkylammonium halides, and alkylpyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylene polypropylene copolymers, (d) amphoteric detergents such as, for example, alkylbeta-aminopropionates, and 2-alkylimidazoline quaternary ammonium salts, and (e) mixtures thereof.

Suitable preservatives and buffers can be used in such formulations. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations ranges from about 5% to about 15% by weight.

Topical dosage forms, such as, but not limited to, ointments, creams, pastes, emulsions, containing the nucleic acid molecule of the present disclosure, can be admixed with a variety of carrier materials well known in the art, such as, e.g., alcohols, aloe vera gel, allantoin, glycerine, vitamin A and E oils, mineral oil, PPG2 myristyl propionate, and the like, to form alcoholic solutions, topical cleansers, cleansing creams, skin gels, skin lotions, and shampoos in cream or gel formulations. Inclusion of a skin exfoliant or dermal abrasive preparation may also be used. Such topical preparations may be applied to a patch, bandage or dressing for transdermal

delivery or may be applied to a bandage or dressing for delivery directly to the site of a wound or cutaneous injury.

The compound(s) of the present disclosure can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. Such liposomes may also contain monoclonal antibodies to direct delivery of the liposome to a particular cell type or group of cell types.

The compound(s) of the present disclosure may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include, but are not limited to, polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropylmethacryl-amidephenol, polyhydroxyethylaspartamidephenol, or polyethyl-eneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

Furthermore, the sialic acid or sialic acid precursors disclosed herein can be administered as a food supplement or incorporated into food or drink items.

RESULTS

In the following results, the methods used were those methods specified in the Methods section of the present disclosure and the references cited therein.

Upregulation of podocyte Angptl4 expression in experimental and human glomerular disease

Angptl4 mRNA expression is upregulated (70-fold) in rat glomeruli at the peak of complement- and leukocyte-independent heterologous phase proteinuria 24 hours after injection of NTS (FIG. 1A and 1B). Injection of NTS into Angptl4 -/- mice caused significant reduction in proteinuria (FIG. 1C) and foot process effacement (FIG. 1D), suggesting a key role for Angptl4 in glomerular disease. Normal rat glomeruli express Angptl4 in a capillary loop pattern that co-localized with podocyte protein CD2AP (FIG. 1E). Further studies revealed early (Day 3, before the onset of proteinuria) and progressive upregulation of Angptl4 mRNA expression in young rats following intravenous injection of a single dose of puromycin aminonucleoside (PAN model) (FIG. 1F), and *in situ* hybridization

confirmed upregulation in a peripheral capillary loop pattern (data not shown) without concomitant change in the proximal tubular signal. In passive Heymann nephritis, a smaller increase in Angptl4 expression was noted, starting after the onset of proteinuria (FIG. 1F). By contrast, Angptl4 mRNA expression did not change in anti-Thy1.1 nephritis, or in collapsing focal and segmental glomerulosclerosis (FIG. 1F) induced in rats by injection of sera from patients with this disease (18). Angptl4 protein expression increased dramatically in podocytes (FIG. 1G) following induction of PAN, with substantial additional overlap with the glomerular basement membrane (GBM), which was confirmed by immunogold electron microscopy (EM) (FIG. 1H). Biopsies from patients with glucocorticoid sensitive MCD and age and sex-matched controls (FIG. 1I) revealed a faint podocyte pattern in control kidney biopsies, and increased expression in the podocyte with additional GBM overlap and trace spotty overlap with the endothelium at the margins.

Podocyte specific overexpression of Angptl4 results in selective proteinuria and podocyte foot process effacement

A transgenic rat models for podocyte specific Angptl4 overexpression was developed and is shown in FIG. 2A (NPHS2-Angptl4 TG). Analysis of mRNA expression in organs that normally express Angptl4 confirmed specificity of expression (FIG. 2B). Histological assessment of 3 month old heterozygous male NPHS2-Angptl4 TG rats revealed normal appearing glomeruli with prominent podocytes (FIG. 2C) on light microscopy and increased podocyte Angptl4 expression by confocal imaging (FIG. 2D). Electron microscopy of 5 month old homozygous and most heterozygous TG rats (FIG. 2E) revealed diffuse foot process effacement. Immunogold EM of homozygous TG rats revealed a correlation between accumulation of Angptl4 in the GBM and progressive development of foot process effacement between age one to five months (FIG. 2F). Both founder lines of NPHS2-Angptl4 TG rats developed significant albuminuria. Female homozygous and male heterozygous rats developed albuminuria as early as age 1 month (FIGS. 3A-3C). Homozygous females developed up to 100-fold, heterozygous males up to 20-fold and homozygous males over 500-fold increase in albuminuria over time. Heterozygous females were not albuminuric. Over 90% of the urinary protein comprises of intact albumin (FIG. 3D), thereby making these rats the first model for selective proteinuria. Immunogold EM using anti-V5 antibody to specifically detect transgene expressed protein revealed gold particles in the podocyte and GBM in

NPHS2-Angptl4 TG rats (FIG. 3E). In keeping with the pro-proteinuric effects of podocyte secreted Angptl4, NPHS2-Angptl4 TG rats develop more proteinuria (FIG. 3F) than wild type littermates after induction of PAN. Blood pressure was significantly lower in proteinuric heterozygous NPHS2-Angptl4 rats compared to wild type controls (data not shown). As also previously published (20), proteinuria in PAN is partially glucocorticoid sensitive on day 6 (FIG. 3G), and some of this glucocorticoid sensitivity is related to Angptl4 (FIG. 3H).

Characterization of podocyte secreted and recombinant Angptl4

Glomerular protein Western blot typically underestimates Angptl4 production, since the protein is rapidly secreted. Angptl4 was analyzed by 2D electrophoresis and Western blotting (FIG. 4A) in glomeruli from control (upper panel), a PAN model at day 6 (middle panel) and a PAN model at day 6 with glucocorticoid coadministration (lower panel). On 2D gel electrophoresis, most Angptl4 in normal glomeruli migrates as glycosylated low order oligomers at neutral pI (pink arrow; 3), though less prominent spots for both glycosylated intact 70 KDa and cleaved (red arrow; 1) and non glycosylated 45 KDa monomeric forms (yellow arrow; 2) were also noted (FIGS. 4A and 4B). In PAN, both neutral pI oligomers, that are reactive with sialic acid binding lectin Maackia amurensis (MAA), and high pI oligomers, that are not MAA reactive, were increased (pink arrow, 3 and orange arrow, 4) (FIGS. 4A and 4B). This increase was blunted in PAN treated with glucocorticoids, though disproportionately higher amounts of high pI oligomers were noted. Densitometric analysis of the Western blots is shown in FIG. 4B.

FIG. 5A shows HEK293 cells stably transfected with a pcDNA-rat Angptl4-V5-His expression construct line showed 55,000 fold upregulation of Angptl4 mRNA expression compared to a control empty vector cell line. *** $P < 0.001$

FIG. 5B shows Angptl4-HEK293 cells secreted mostly intact protein into the supernatant underserum free conditions (as demonstrated by 2D gel electrophoresis and Western blot with pre-immune serum and anti-Angptl4 antibodies).

Sialylation of Angptl4 was studied in vitro (FIG. 4C) using the Angptl4-HEK293 stable cell line. HEK293 cells normally express no or little Angptl4 polypeptide; after transfection with the pcDNA-rat Angptl4-V5-His expression construct, this cell line showed a 55,000 fold upregulation of Angptl4 mRNA expression compared to a control empty vector cell line (FIG. 5A). Furthermore, the Angptl4 polypeptide expressed by the HEK293-Angptl4 cell line is normally secreted

by this cell line is only trace MAA reactive at the low pI end (FIG. 4C upper panel and upper middle panel, green arrow 1 and FIG. 5B), and is therefore mostly hyposialylated. Incubation of the cell line with sialic acid precursor N-Acetyl-D-mannosamine (ManNAc; FIG. 4C, lower middle panel and lower panel) resulted in a shift of the protein towards neutral pI, increased reactivity to MAA (blue arrow, 2), and therefore, increased sialylation, showing that sialylation plays a key role in the differential electrophoretic migration of Angptl4.

Since an increase in hyposialylated Angptl4 was noted in PAN, the role of this lack of sialylation in the pathogenesis of proteinuria was studied. FIG. 6A shows representative tracing of albuminuria from pilot studies with heavily albuminuric rats receiving increasing doses of ManNAc. The study revealed that ManNAc reduced albuminuria in these animals. However, a large dose requirement to reduce albuminuria in these rats was required. In order to conduct a more efficient and indicative study, rats with 15 -25 fold higher albuminuria than wild type rats were used for further analysis.

NPHS2-Angptl4 TG rats that received ManNAc (1 mg/ml) in tap water (FIG. 4D) had over 40% reduction in albuminuria over a 12 day period compared to baseline, whereas albuminuria in control NPHS2-Angptl4 TG rats receiving tap water increased 2.5 fold over the duration of the study. FIG. 6B shows individual tracings from the study group of NPHS2-Angptl4 TG rats treated with ManNAc (1 mg/ml and which went through the complete study. Two urine collections 12 days apart were conducted prior to the start of the study to ensure that these rats developed increasing albuminuria with time. The study revealed that ManNAc (1 mg/ml) reduced albuminuria in these animals.

Densitometry analysis of Western blots for glomerular Angptl4 from rats euthanized on Day 12 (FIGS. 6C and 6D) showed an increase in the neutral pI Angptl4 fraction from 48.3 + 5% in control rats to 72.9 + 1.4% in ManNAc treated rats (FIG. 6D). The neutral pI fraction was also reactive with sialic acid binding lectin Sambucus nigra (SNA I), confirming increased sialylation of Angptl4 in ManNAc treated rats (FIG. 6C).

FIG. 7 shows improvements in nephrotic syndrome parameters in ManNAc treated rats with PAN, a model of MCD. Rats (n = 5 rats/group) received a single intravenous injection of puromycin aminonucleoside (10 mg/100 gm), and were treated with ManNAC 80 mg/Kg body weight in tap water starting on Day 4, which

coincides with the onset of proteinuria. Data from Day 6 is shown. Significant reduction in proteinuria (FIG. 7A), improvement in plasma albumin levels (FIG. 7B), reduction in cholesterol (FIG. 7C) and reduction in triglycerides (FIG. 7D) were noted in ManNAc treated rats when compared with control PAN rats. These data are consistent with the results described above.

Proteinuria, including albuminuria, has been noted in other diseases as discussed herein, including diabetes mellitus. The role of Angptl4 in diabetes mellitus was also analyzed using the *db/db* mouse. The *db/db* was identified initially in 1966 in Jackson Labs as an obese mouse that was hyperphagic soon on weaning. The diabetic gene (*db*) is transmitted as an autosomal recessive trait. The *db* gene encodes for a G-to-T point mutation of the leptin receptor, leading to abnormal splicing and defective signaling of the adipocyte-derived hormone leptin. Lack of leptin signaling in the hypothalamus will lead to persistent hyperphagia and obesity with consequently high leptin and insulin levels. Several studies established that albumin excretion rates are higher by 8- to 62-fold in *db/db* mice beginning at the age of 8 wk. The range of albuminuria is between 68 and 303 μg/24 h in the *db/db* male mouse, whereas it is between 4 and 21 μg/24 h in the age-matched heterozygous littermate.

FIGS. 8A-C show the effect of ManNAc therapy on proteinuria in diabetic animals. FIG. 8A shows 2D gel electrophoresis and Western blot analysis of glomeruli from diabetic *db/db* and control *db/m* mice. This analysis showed increased expression of Angptl4 in diabetic *db/db* mouse glomeruli, including the high pI hyposialylated forms that are involved in the pathogenesis of proteinuria. These high pI hyposialylated forms of Angptl4 are sensitive to sialic acid precursor therapy, including ManNAc and ManNAc derivatives.

FIG. 8B shows 2D gel electrophoresis and Western blot analysis of glomeruli from Zucker diabetic fatty rats. This analysis showed increased expression of Angptl4 in Zucker diabetic fatty rat glomeruli, including the high pI hyposialylated forms that are involved in the pathogenesis of proteinuria. These high pI hyposialylated forms of Angptl4 are sensitive to sialic acid precursor therapy, including ManNAc and ManNAc derivatives. FIG. 8C shows treatment with a sialic acid precursor reduced proteinuria in this model. In FIG. 8C, 13 week old male Zucker diabetic fatty rats were treated with ManNAc in tap water or with plain tap water (n = 4 rats/group). Significant reduction in proteinuria was noted in the

ManNAc treated group for up to 20 days at 70 mg/Kg and at an increased dose of 140 mg/Kg.

These results show that administration of sialic acid precursors treats diabetes mellitus through restoration of the normal sialyation of Angptl4. As shown in FIG. 8C, administration of sialic acid precursors reduces proteinuria observed in diabetes mellitus through restoration of normal sialylation of the Angptl4 polypeptide as described herein.

The present disclosure shows a key role of podocyte secreted Angptl4 in the pathogenesis of nephrotic syndrome, exemplified using a model of MCD and the role of Angptl4 in conditions related to nephrotic syndrome, such as, but not limited to, proteinuria. The present disclosures shows increased podocyte expression of Angptl4 in human kidney biopsies, selective proteinuria with over 500-fold increase in albuminuria in NPHS2-Angptl4 TG rats, glucocorticoid sensitivity of the Angptl4 gene, and light and electron microscopic findings consistent with human MCD. The role of Angptl4 in the development of proteinuria and foot process effacement was further confirmed by the significant reduction in proteinuria and foot process effacement in Angptl4 -/- mice injected with NTS. The absence of a reliable model of MCD in mice necessitated the use of NTS.

The secretion of high pI Angptl4 by the podocyte in MCD is likely to facilitate the tethering of Angptl4 to the GBM, and prior studies have shown the binding of Angptl4 to heparan sulfate proteoglycans (21). This correlates well with reduced GBM charge, a hallmark of MCD, in rodents with TG expression of Angptl4 from the podocyte. It is likely that charge facilitates the transit of high pI Angptl4 across the GBM against the direction of fluid flow. Changes in sialylation explain the bulk of the variations in pI and the biological role of Angptl4 in proteinuria, since the active oligomeric forms of Angptl4 secreted from podocytes were not threonine phosphorylated, and increasing the sialylation of Angptl4 reduced proteinuria. There are two predicted O-and *N*- glycosylation sites each in rat and human Angptl4 where sialic acid residues could be incorporated. Treatment with sialic acid precursors constitute a new potential therapeutic tool to reduce proteinuria in nephrotic syndrome, such as, but not limited to, MCD, and perhaps common multisystem disorders like diabetes mellitus, in which Angptl4 plays a role.

Additional, as yet undefined, podocyte or endothelial cell secreted factors probably work synergistically with Angptl4 in the pathogenesis of MCD. In NPHS2-

Angptl4 TG rats, podocyte secreted Angptl4 remains tethered to the glomerular capillary loop or escapes into the urinary space, where it is taken up by the proximal tubule. After induction of low dose PAN in these rats, which provides these additional permeabilizing factors, podocyte secreted Angptl4 escapes into the circulation. The lack of additional permeabilizing factors in NPHS2-Angptl4 TG rats is also likely to also explain the more gradual onset of proteinuria than in PAN or patients with MCD.

The foregoing shows that administration of a sialic acid precursor restores normal sialylation of Angpl4 polypeptide and reduces physiological abnormalities associated nephrotic syndrome (including, but not limited to, MCD, FSGS, MN/MGN, MPGN or diabetic nephropathy), diabetes mellitus, lupus nephritis or primary glomular disease. As a result, the present disclosure provides a treatment for such conditions.

METHODS

Cloning of full length rat Angptl4, and generation of antibody against full length recombinant Angptl4

The full length rat Angptl4 open reading frame of 1218 bp from our previous experiments (7), excluding the stop codon, was cloned into pcDNA3.1/V5-HisB for eukaryotic expression, and into pET28a for prokaryotic expression. The E. Coli expressed purified full length protein was used to generate a polyclonal antibody in rabbits (Proteintech group, Inc. Chicago IL USA) that was tested by ELISA and Western blot. Antibody reactive bands were excised from GelCode blue stained gels, trypsin digested and presence of Angptl4 peptide sequences confirmed by MALDI-TOF/TOF. Part of the antiserum was affinity purified to the antigen. Unless otherwise specified, all studies described used this antibody. An additional polyclonal antibody against the N-terminal part of rat Angptl4 (amino acids 7 - 86 excluding signal peptide) was similarly raised in rabbits.

Induction of proteinuria in animal models of human glomerular disease

All animal studies were approved by the institutional IACUC. Induction of animal models of proteinuria (n = 4 rats/group) in WT rats are described in previous publications in parenthesis: PAN (7), PFIN (7), PAN with glucocorticoids (20), non-HIV collapsing glomerulopathy (18), nephrotoxic serum induced heterologous phase proteinuria (7. Anti-Thyl . 1 nephritis was induced by injection of 200 meg of anti-Thyl . 1 (Ox-7 hybridoma) or

control IgG IV into different groups of male Wistar rats (100-125 gm, n = 4 rats/group), and rats euthanized after 24 and 72 hours.

The following techniques are described in prior publications: Taqman real time PCR (26), confocal imaging (7), in situ hybridization (27), promoter - reporter studies (7), immunogold EM (26), glomerular extraction and processing for Western blot (26), assessment of charge by PEI method (28). For alcian blue staining, the pH of the staining solution was adjusted to 2.5 using acetic acid, and 0.1% nuclear fast red solution was used as a counterstain. Densitometry of glomerular basement membrane alcian blue stain (20 glomeruli/rat, 3 rats/group) was assessed using Image-Pro software (Media Cybernetics, Inc., Bethesda MD, USA). Densitometry of 2D gel Western blots was assessed using Gel-Pro Analyzer software (Media Cybernetics, Inc.). Taqman real time PCR primers and probes are listed in Table 1. For in situ hybridization, the digoxigenin labeled probe for rat Angptl4 included bp 1 to 548 of the ORF.

To obtain samples for post heparin LPL activity, rats were injected intravenously with 10 units / 100 gm weight of porcine heparin 15 minutes prior to euthanasia, and activity measured using an assay from Roar Biomedical, Inc (New York NY). Serum triglycerides were measured in the fasting state.

Injection of NTS into Angptl4 -/- mice

Angptl4 -/- mice were provided to Sander Kersten as a kind gift from Eli Lily Corporation (Indianapolis IN USA). The study protocol was approved by the Animal Studies Committee at Wageningen University. Eleven week old male Angptl4 -/- or +/+ mice (n = 4 mice / group) were injected intravenously with 1.5 mg y2-NTS or normal sheep serum (Sigma Aldrich St. Louis MO USA), spot urine samples collected at 48 hours, mice euthanized at 72 hours, plasma collected for biochemical measurements, and kidneys preserved for histological analysis. Urine albumin was assessed by ELISA (Bethyl laboratories, Montgomery TX USA) and urine creatinine measured by mass spectrometry. To assess for foot process effacement, the mean width of foot processes was first measured in control treated Angptl4 +/+ mouse transmission electron micrographs (10 equally spaced readings/loop, 3 loops/glomerulus, 3 glomeruli/kidney, 3 kidneys/group). Effacement was described as an over 2.5 fold increase in mean width. Total and effaced foot processes were counted in NTS treated or control treated Angptl4 -/- mice.

Studies with archived human samples

Immunostaining of archived human kidney biopsies (n = 5 biopsies per condition) was conducted on samples obtained via IRB approved protocols at the Instituto Nacional de Cardiologia, Mexico City. Control kidney biopsies used for these studies were sex and age matched protocol pre-transplant biopsies. Archival human sera for 2D gel electrophoresis and Western blot (n= 4 samples/condition) were obtained from a previously published study (29).

Generation of transgenic rats:

NPHS2-Angptl4 TG rats were generated as follows: The vector pTRE-tight was digested with StuI and EcoRI to remove the minimum CMV promoter between bp 278 and 324, the 5' overhangs blunt ended with T4 DNA polymerase, and re-ligated to generate pTRE-tight MP (minus promoter). For podocyte specific expression, a rat Angptl4 cDNA construct (including the signal sequence) with a C-terminal V5 tag was placed upstream of a SV40 polyA tail. The human NPHS2 promoter was cloned upstream by PCR using a published human NPHS2 promoter construct as template (gi22652661) without DMSO to exclude a naturally occurring loop between bps 2343 and 2568 to improve expression.

Transgenic rats were generated by microinjection of the digested DNA constructs into fertilized Sprague Dawley eggs (conducted at University of Michigan), implantation into pseudopregnant host Sprague Dawley females, and the resulting offsprings were genotyped by routine PCR and TaqMan genomic DNA real time PCR strategy using construct specific and control genomic prolactin primer and probe combinations. Two founder lines for podocyte specific expression were generated. Data from NPHS2-Angptl4 TG rat line 740 (5 copies of the transgene) and were stable over 4 generations, are presented. Urinary total protein was assessed using the Bradford method (Biorad laboratories, Hercules CA USA), and albuminuria by ELISA (Bethyl laboratories, Montgomery TX USA).

Measurement of rat blood pressure

Blood pressure and pulse rate were measured in six 5 month old wild type and proteinuric heterozygous NPHS2-Angptl4 TG rats by the tail cuff method using the SC-1000 apparatus from Hetteras Instruments, Inc. (Cary NC USA). A minimum of 80 reading were analyzed per group.

Development and characterization of a stable cell line and recombinant Angptl4 protein A stable cell line was developed using a rat Angptl4 pcDNA 3.1-

V5/His construct, along with control empty vector stable cell lines. The Angptl4 stable cell line develops 55,253 $\pm$ 5,155 fold rat Angptl4 mRNA upregulation (over undetectable baseline as reference value of 1) and secretes a 70 kDa protein in serum free conditions and a 55 kDa protein in the presence of serum. The full length V5-His tagged protein that was affinity purified from serum free media using a Nickel affinity column. Western blot studies performed on 2D gels revealed that most of the recombinant Angptl4 migrated as a high isoelectric point (8.3-8.5) protein, and was non-phosphorylated.

Promoter – reporter studies.

The mouse Angptl4 promoter previously published (31) had a transcriptional start site at -183 bp upstream of ATG in the liver. We conducted 5' RACE and primer extension analysis using mouse kidney mRNA and confirmed the same transcriptional start site in the kidney. Next, a 2 Kb fragment upstream of the transcriptional start site was clone using BAC clone RP23-27D5 as a template into pSEAP2 basic. A 2 Kb human Angptl4 promoter constructs were similarly generated using BAC clone RP11-886P16.

Incubation of HEK 293 cells and GECs transfected with Angptl4-pSEAP or empty vector promoter – reporter constructs for mouse Angptl4 with dexamethasone (10^{-3}M) show a decline in SEAP activity at 48 hours. This decline in activity is mediated via the glucocorticoid receptor, since it is reversed by the receptor antagonist mifepristone ($2x10^{-5}$M). Glucocorticoid sensitivity of the human promoter was also assessed (dexamethasone 10^{-4}M). All transfection reactions were normalized using the pβgal vector (Clontech, Mountain View CA USA).

In vitro and in vivo studies with ManNAc

To study the effect of manNAc on the sialylation of recombinant protein, the Angptl4-HEK293 stable or pcDNA3.1-HEK293 control stable cell lines were grown to confluence in 15 cm dishes, washed twice with warm PBS, then incubated with serum free DMEM without phenol red with or without 25 mM manNAc for 48 hours, after which the media was harvested, concentrated, protein assay conducted, and loaded on the 2D gels (200 μg / gel). Western blots of glomeruli or recombinant protein were conducted using rabbit anti-Angptl4 (full length) antibody, preimmune rabbit serum and lectins SNA I – HRP and MAA – HRP (E-Y laboratories, San Mateo CA USA).

Subsequent in vitro studies with neutral pI Angptl4 (ManNAc treated) or high pI Angptl4 (untreated) used either His tag purified protein or concentrated supernatant harvested from Angptl4-HEK293 stable in a same manner. Control protein was concentrated from control stable cell line supernatant.

Pilot studies in heavily albuminuric NPHS2-Angptl4 rats revealed a large ManNAc dose requirement (8 mg/ml tap water) to maintain 20 – 30 % reduction in albuminuria. To make the study affordable, 7 homozygous male NPHS2-Angptl4 rats, age 3 – 3.5 months, with 15 – 25 fold higher albuminuria than wild type rats, were given ManNAc 1 mg/ml of tap water for 12 days (ManNAc phase), after which three were euthanized and the others returned to plain tap water for another 24 days (washout phase). 18 hour urine collections were done twice (Day -12 and Day 0) before the study to confirm rising albuminuria, and periodically during the study. Another seven male NPHS2-Angptl4 rats of similar age were assessed for baseline albuminuria levels, given normal tap water (control group), three euthanized on Day 12 and others followed up to day 36, after which albuminuria was reassessed and the animals euthanized. Daily water intake of each rat was charted. At each euthanasia time point, kidneys were removed after perfusion, glomeruli isolated and processed for 2D gel electrophoresis. Albuminuria on Day 0 for each rats was denoted as 100% and all subsequent albuminuria values were expressed relative to Day 0.

Statistical analysis

Analysis of difference in proteinuria or gene expression involving three or more groups was conducted by ANOVA with post analysis testing using GraphPad InStat software, Version 3.05. For comparison of two groups, the unpaired Students t test in Microsoft Excel 2003 was used.

The foregoing description illustrates and describes the methods and other teachings of the present disclosure. Additionally, the disclosure shows and describes only certain embodiments of the methods and other teachings disclosed, but, as mentioned above, it is to be understood that the teachings of the present disclosure are capable of use in various other combinations, modifications, and environments and is capable of changes or modifications within the scope of the teachings as expressed herein, commensurate with the skill and/or knowledge of a person having ordinary skill in the relevant art. The embodiments described hereinabove are further intended to explain best modes known of practicing the methods and other teachings of the present disclosure and to enable others skilled in the art to utilize the teachings of the

present disclosure in such, or other, embodiments and with the various modifications required by the particular applications or uses. Accordingly, the methods and other teachings of the present disclosure are not intended to limit the exact embodiments and examples disclosed herein.

Table 1- List of primers and probes used for Taqman real time PCR

Gene / transgene	Forward primer	Reverse primer	Taqman probe
Angptl4	tctgggatctccaccatttttg	tcaccgtccagcctccat	caactgtgagatgacttc
Angptl4	cgccacccgcttacaca	cagaggctggatctggaaaagt	tgccaggaactcttt
NPHS2-Angptl4 construct	tacaggctaccaccctgttgatc	aaccgcgggccctctag	ccatggaggctacagca
Prolactin (genomic)	cttgaagggattgaaaagataattagc	ccatgagtcagaaaagcattgaac	aggtgagcattttcctg

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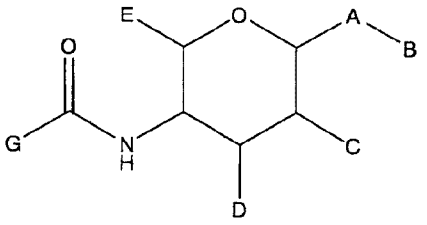
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Claims

1. Use of a sialic acid, a sialic acid precursor or a combination of the foregoing in the manufacture of a medicament for the treatment or prevention of diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease in a subject, wherein the diabetic nephropathy, diabetes mellitus, lupus nephritis or primary glomular disease is characterized by the presence of a hyposialylated form of Angptl4, the subject has a non-reduced level of sialic acid production, and the sialic acid precursor is N-levulinoylmannosamine or a compound of the structure:



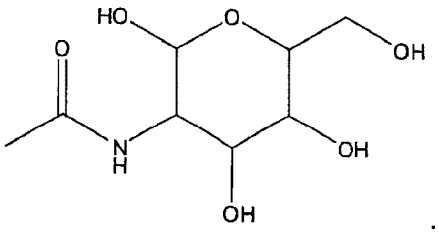
wherein:

A is CH_2 or NH;

B, C, D and E are each independently selected from the group consisting of: H, OH, X, O-CO-X and O-X, wherein X is an unsubstituted alkyl or alkenyl, X being selected independently for each group B, C and D; and

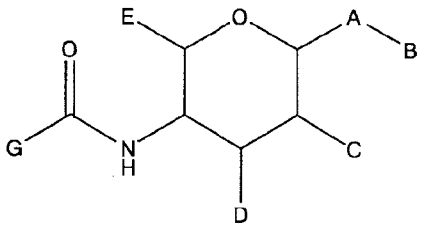
G is H, OH, Y or O-Y, wherein Y is an unsubstituted alkyl or alkenyl.

2. The use of claim 1, wherein the sialic acid precursor has the structure



3. The use of claim 1, wherein X and Y are each independently a C1 to C5 alkyl.
4. The use of claim 1, wherein the sialic acid precursor is N-acetyl-d-mannosamine, Bu4ManNAc, 3,4,6-O-Bu3ManNAc, or 1,3,4-O-Bu3ManNAc.
5. The use of claim 1, wherein the sialic acid compound is in a therapeutically effective amount.
6. The use of claim 1, wherein the use restores sialylation of Angptl4.

7. Use of a sialic acid, a sialic acid precursor or a combination of the foregoing in the manufacture of a medicament for the treatment or prevention of minimal change disease in a subject, wherein the minimal change disease is characterized by the presence of a hyposialylated form of Angptl4, the subject has a non-reduced level of sialic acid production, and the sialic acid precursor is N-levulinoylmannosamine or a compound of the structure:



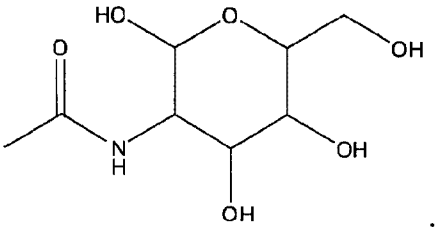
wherein:

A is CH_2 or NH;

B, C, D and E are each independently selected from the group consisting of: H, OH, X, O-CO-X and O-X, wherein X is an unsubstituted alkyl or alkenyl, X being selected independently for each group B, C and D; and

G is H, OH, Y or O-Y, wherein Y is an unsubstituted alkyl or alkenyl.

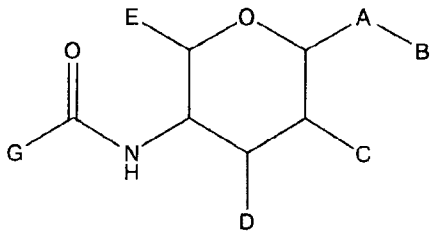
8. The use of claim 7, wherein the sialic acid precursor has the structure



9. The use of claim 7, wherein X and Y are each independently a C1 to C5 alkyl.
10. The use of claim 7, wherein the sialic acid precursor is N-acetyl-d-mannosamine, Bu4ManNAc, 3,4,6-O-Bu3ManNAc, or 1,3,4-O-Bu3ManNAc.
11. The use of claim 7, wherein the sialic acid compound is in a therapeutically effective amount.
12. The use of claim 7, wherein the use reduces edema, reduces proteinuria, increases plasma albumin levels, reduces hypercholesterolemia, reduces hypertriglyceridemia or a combination of the foregoing.

13. The use of claim 7, wherein the use restores sialylation of Angptl4.

14. Use of a sialic acid, a sialic acid precursor or a combination of the foregoing in the manufacture of a medicament for increasing the sialylation of at least a portion of Angltp4 in the subject, wherein the subject has an increased level of Angptl4 and the sialic acid precursor is N-levulinoylmannosamine or a compound of the structure:



wherein:

A is CH_2 or NH;

B, C, D and E are each independently selected from the group consisting of: H, OH, X, O-CO-X and O-X, wherein X is an unsubstituted alkyl or alkenyl, X being selected independently for each group B, C and D; and

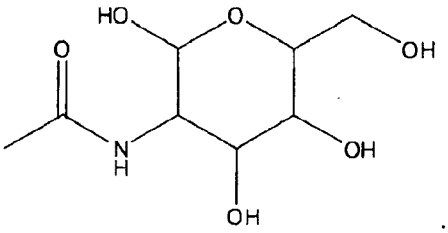
G is H, OH, Y or O-Y, wherein Y is an unsubstituted alkyl or alkenyl.

15. The use of claim 14, wherein the increased level of Angptl4 causes at least a portion of the Angptl4 in the subject to be in the hyposialylated state.

16. The use of claim 14, wherein the hyposialylated state increases proteinuria.

17. The use of claim 14, wherein increasing the sialylation of at least a portion of Angptl4 in the subject reduces proteinuria.

18. The use of claim 14, wherein the sialic acid precursor has the structure



19. The use of claim 14, wherein X and Y are each independently a C1 to C5 alkyl.

20. The use of claim 14, wherein the sialic acid precursor is N-acetyl-d-mannosamine, Bu4ManNAc, 3,4,6-O-Bu3ManNAc, or 1,3,4-O-Bu3ManNAc.

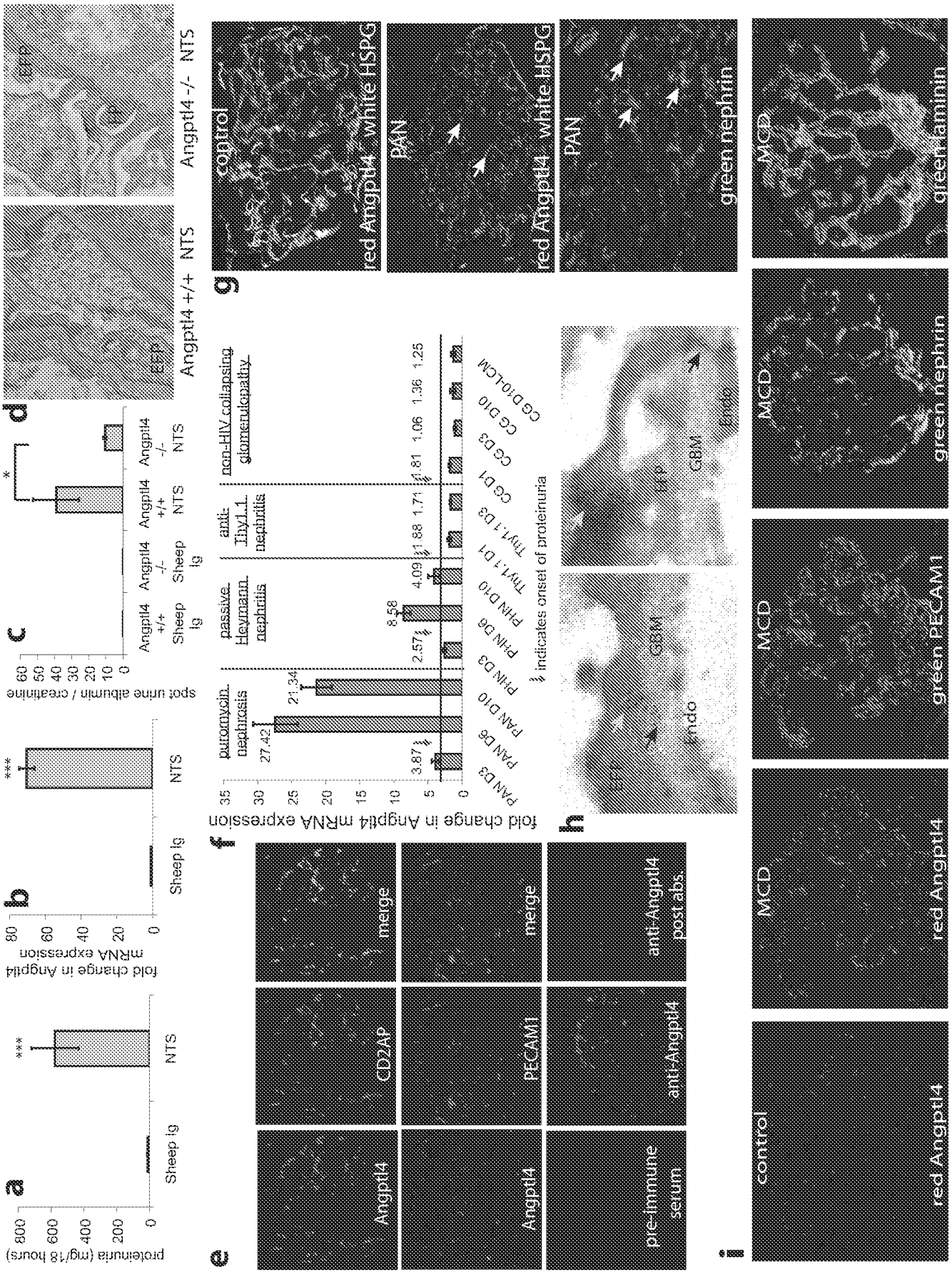

a
proteinuria (mg/18 hours)
Sheep Ig
NTS
b
fold change in Angptl4 mRNA expression
Sheep Ig
NTS
c
spot urine albumin / creatinine
Angptl4 +/+ Sheep Ig
Angptl4 -/- Sheep Ig
Angptl4 +/+ NTS
Angptl4 -/- NTS
d
EFP
Angptl4 +/+ NTS
EFP
Angptl4 -/- NTS
e
Angptl4
pre-immune serum
CD2AP
PECAM1
merge
anti-Angptl4
anti-Angptl4 post abs.
f
fold change in Angptl4 mRNA expression
puromycin nephrosis
passive Heymann nephritis
anti-Thy1.1 nephritis
non-HIV collapsing glomerulopathy
indicates onset of proteinuria
PAN D3
PAN D6
PAN D10
PHN D3
PHN D6
PHN D10
TNT 1 D1
TNT 1 D3
CG D1
CG D3
CG D10
CG D10LCM
g
control
red Angptl4
white HSPG
PAN
green nephrin
green laminin
h
EFP
GBM
Endo
i
control
red Angptl4
MCD
green PECAM1

Figure 1

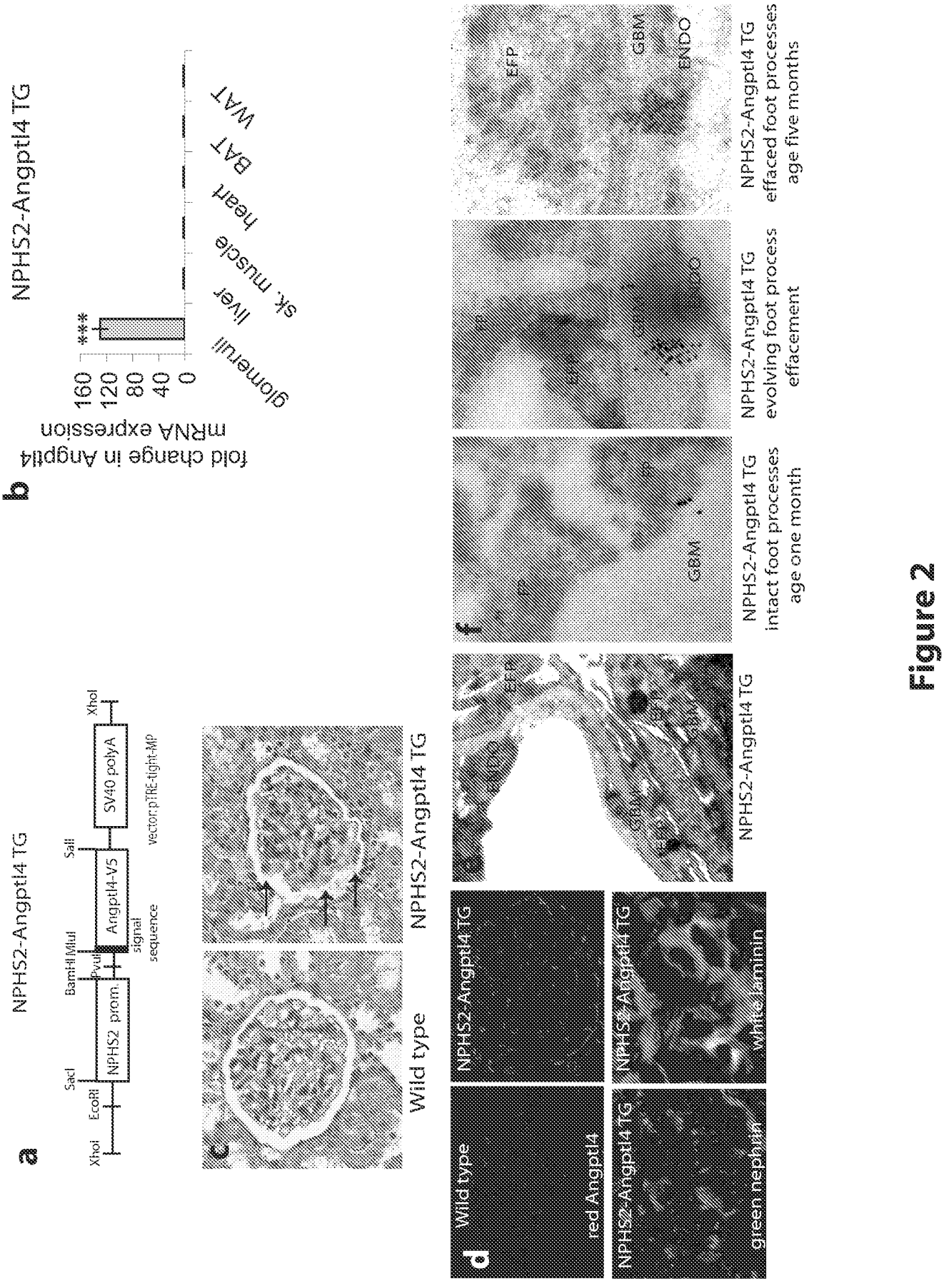

a
NPHS2-Angptl4 TG
XhoI
EcoRI
SacI
NPHS2 prom.
PvuI
BamHI
MluI
Angptl4-V5
signal sequence
SalI
SV40 polyA
XhoI
vector:pTRE-tight-MP
b
NPHS2-Angptl4 TG
fold change in Angptl4 mRNA expression
***
glomeruli
liver
sk. muscle
heart
BAT
WAT
c
Wild type
NPHS2-Angptl4 TG
d
Wild type
red Angptl4
NPHS2-Angptl4 TG
green nephrin
NPHS2-Angptl4 TG
white laminin
e
EFP
GBM
NPHS2-Angptl4 TG
f
FP
GBM
NPHS2-Angptl4 TG
intact foot processes
age one month
FP
GBM
ENDO
NPHS2-Angptl4 TG
evolving foot process effacement
EFP
GBM
ENDO
NPHS2-Angptl4 TG
effaced foot processes
age five months

Figure 2

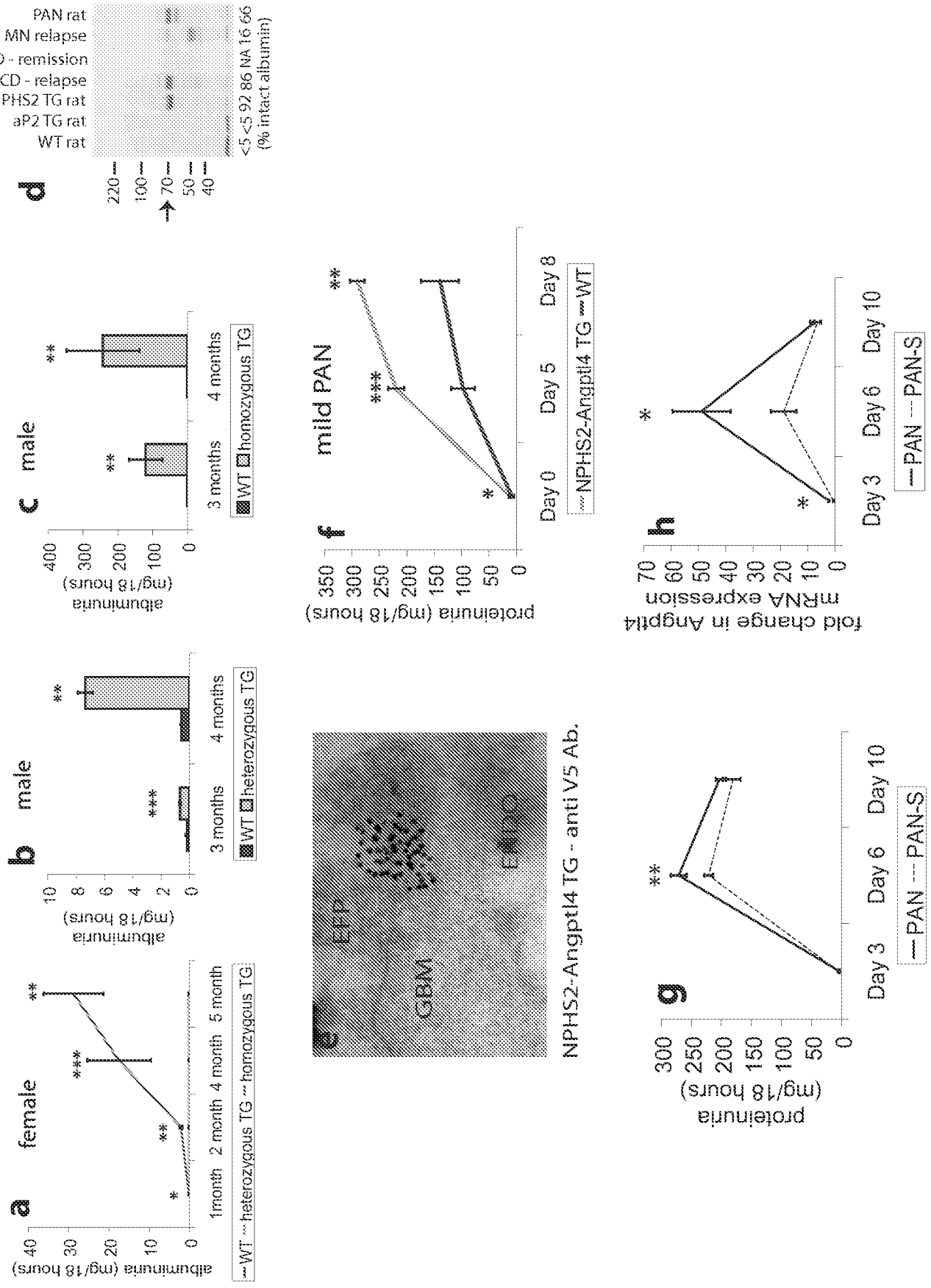

a female
albuminuria (mg/18 hours)
1month 2 month 4 month 5 month
WT heterozygous TG homozygous TG
b male
albuminuria (mg/18 hours)
3 months 4 months
WT heterozygous TG
c male
albuminuria (mg/18 hours)
3 months 4 months
WT homozygous TG
d
PAN rat
MN relapse
MCD - remission
MCD - relapse
NPHS2 TG rat
aP2 TG rat
WT rat
<5 <5 92 86 NA 16 66
(% intact albumin)
e
EFT
GBM
FP
NPHS2-Angptl4 TG - anti V5 Ab.
f mild PAN
proteinuria (mg/18 hours)
Day 0 Day 5 Day 8
NPHS2-Angptl4 TG WT
g
proteinuria (mg/18 hours)
Day 3 Day 6 Day 10
PAN PAN-S
h
fold change in Angptl4 mRNA expression
Day 3 Day 6 Day 10
PAN PAN-S

Figure 3

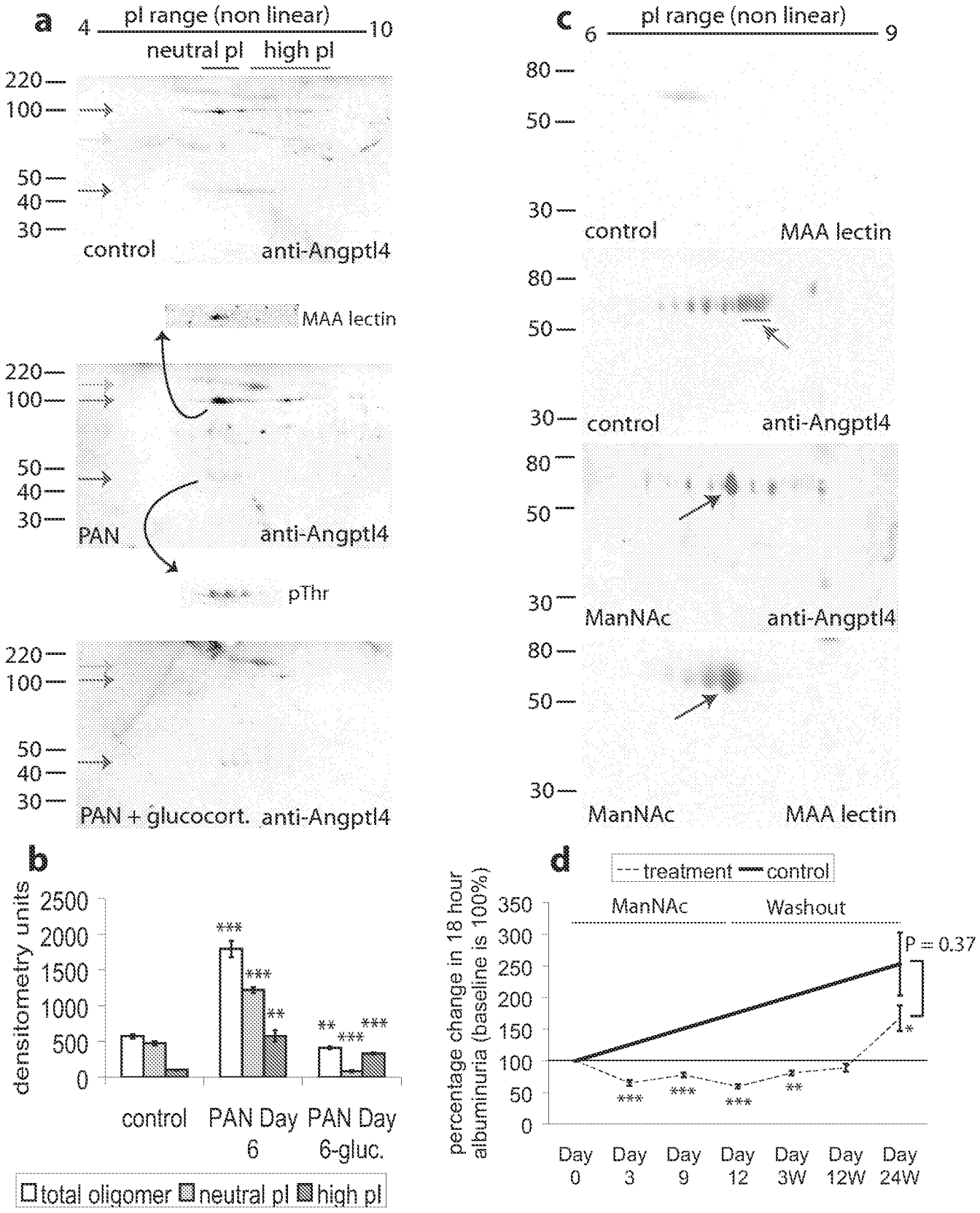

a
pI range (non linear)
4
10
neutral pI
high pI
220
100
50
40
30
control
anti-Angptl4
MAA lectin
PAN
pThr
PAN + glucocort.
b
densitometry units
2500
2000
1500
1000
500
0
control
PAN Day 6
PAN Day 6-gluc.
total oligomer
neutral pI
high pI
c
pI range (non linear)
6
9
80
50
30
control
MAA lectin
anti-Angptl4
ManNAc
d
treatment
control
ManNAc
Washout
P = 0.37
percentage change in 18 hour albuminuria (baseline is 100%)
350
300
250
200
150
100
50
0
Day 0
Day 3
Day 9
Day 12
Day 3W
Day 12W
Day 24W

Figure 4

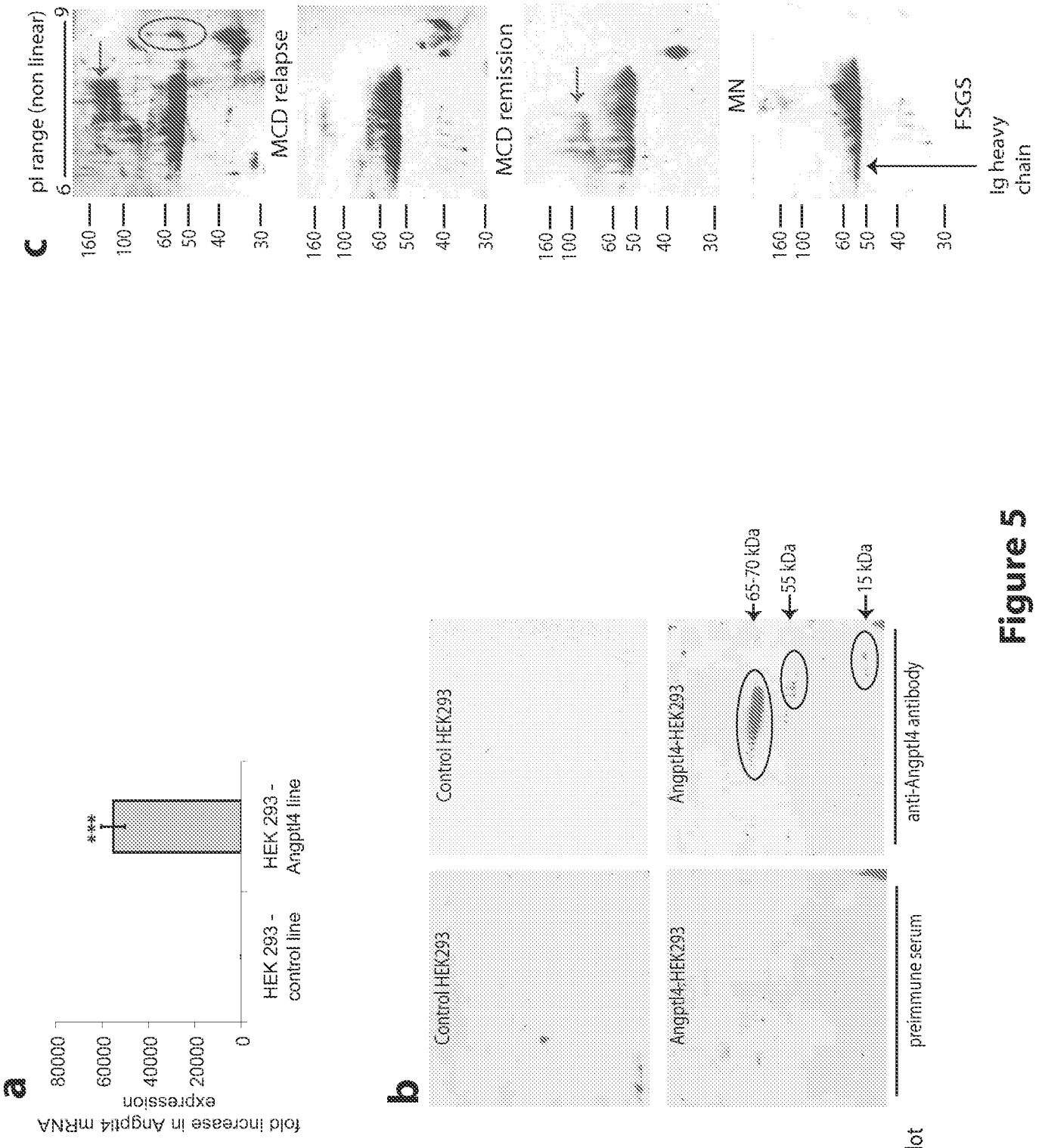

a
fold increase in Angptl4 mRNA expression
80000
60000
40000
20000
0
***
HEK 293 - control line
HEK 293 - Angptl4 line
b
Control HEK293
Angptl4-HEK293
Western blot
preimmune serum
anti-Angptl4 antibody
65-70 kDa
55 kDa
15 kDa
c
pI range (non linear)
6
9
MCD relapse
MCD remission
MN
FSGS
Ig heavy chain
160
100
60
50
40
30

Figure 5

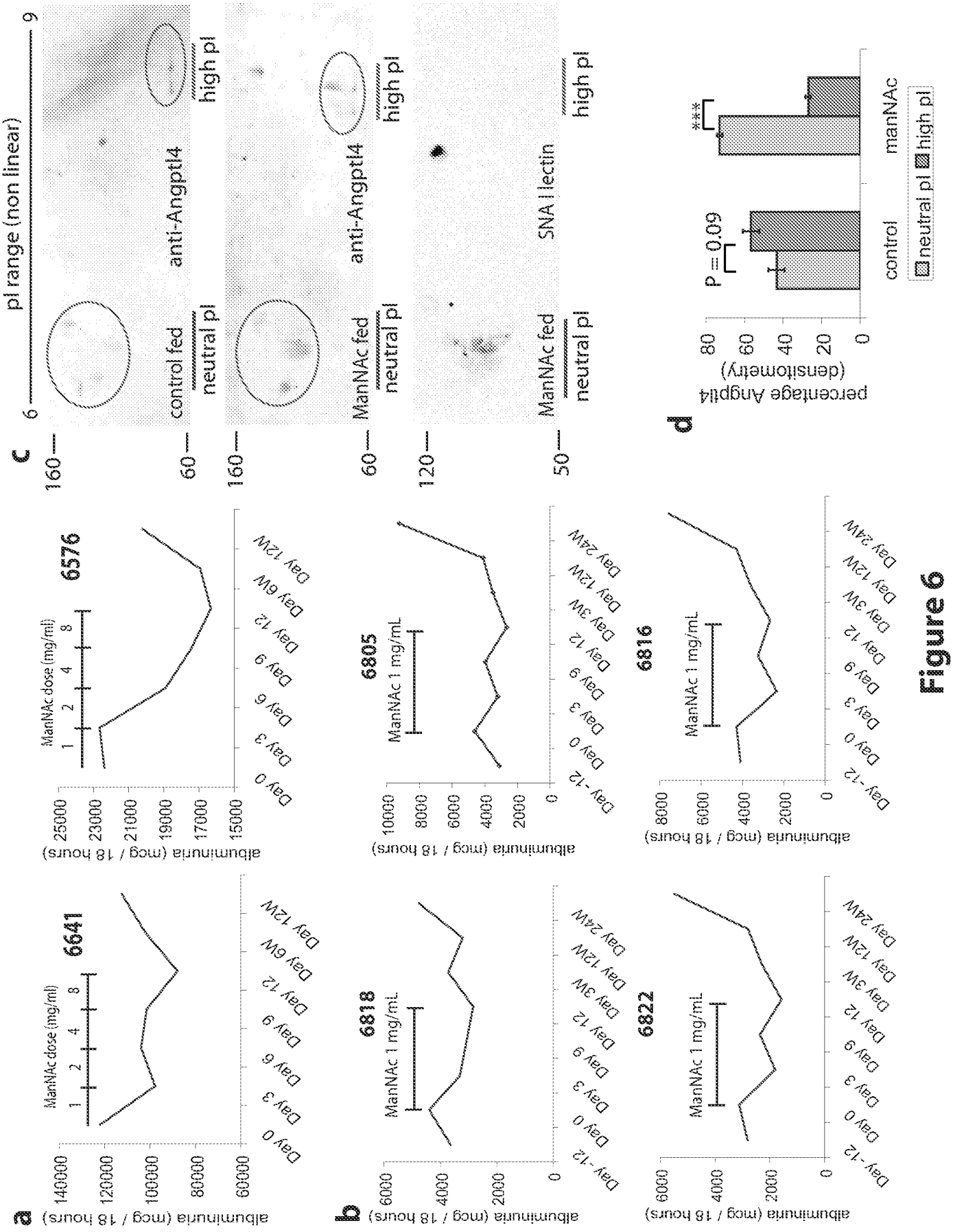

a
b
c
d
albuminuria (mcg / 18 hours)
ManNAc dose (mg/ml)
6641
6818
6822
6576
6805
6816
ManNAc 1 mg/mL
Day 0
Day 3
Day 6
Day 9
Day 12
Day 6W
Day 12W
Day -12
Day 3W
Day 12W
Day 24W
pI range (non linear)
control fed
neutral pI
anti-Angptl4
high pI
ManNAc fed
SNA I lectin
percentage Angptl4 (densitometry)
P = 0.09
***
control
manNAc
neutral pI
high pI

Figure 6

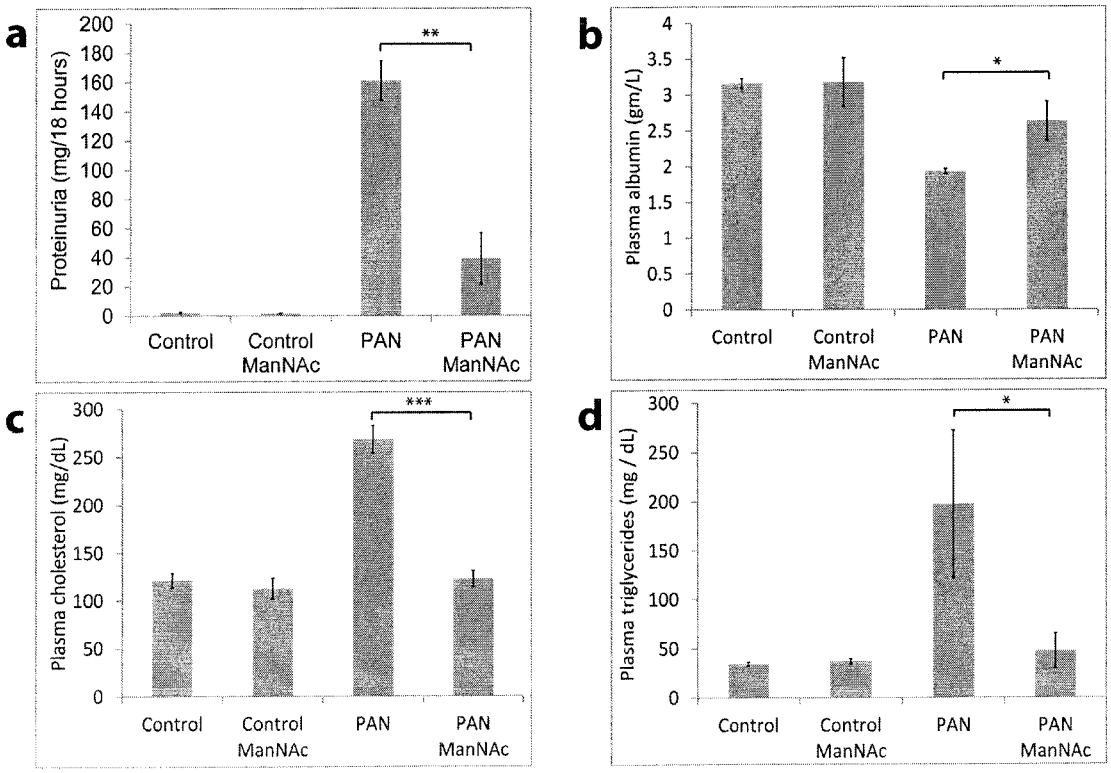

a
Proteinuria (mg/18 hours)
200
180
160
140
120
100
80
60
40
20
0
**
Control
Control ManNAc
PAN
PAN ManNAc
b
Plasma albumin (gm/L)
4
3.5
3
2.5
2
1.5
1
0.5
0
*
Control
Control ManNAc
PAN
PAN ManNAc
c
Plasma cholesterol (mg/dL)
300
250
200
150
100
50
0
***
Control
Control ManNAc
PAN
PAN ManNAc
d
Plasma triglycerides (mg / dL)
300
250
200
150
100
50
0
*
Control
Control ManNAc
PAN
PAN ManNAc

FIGURE 7

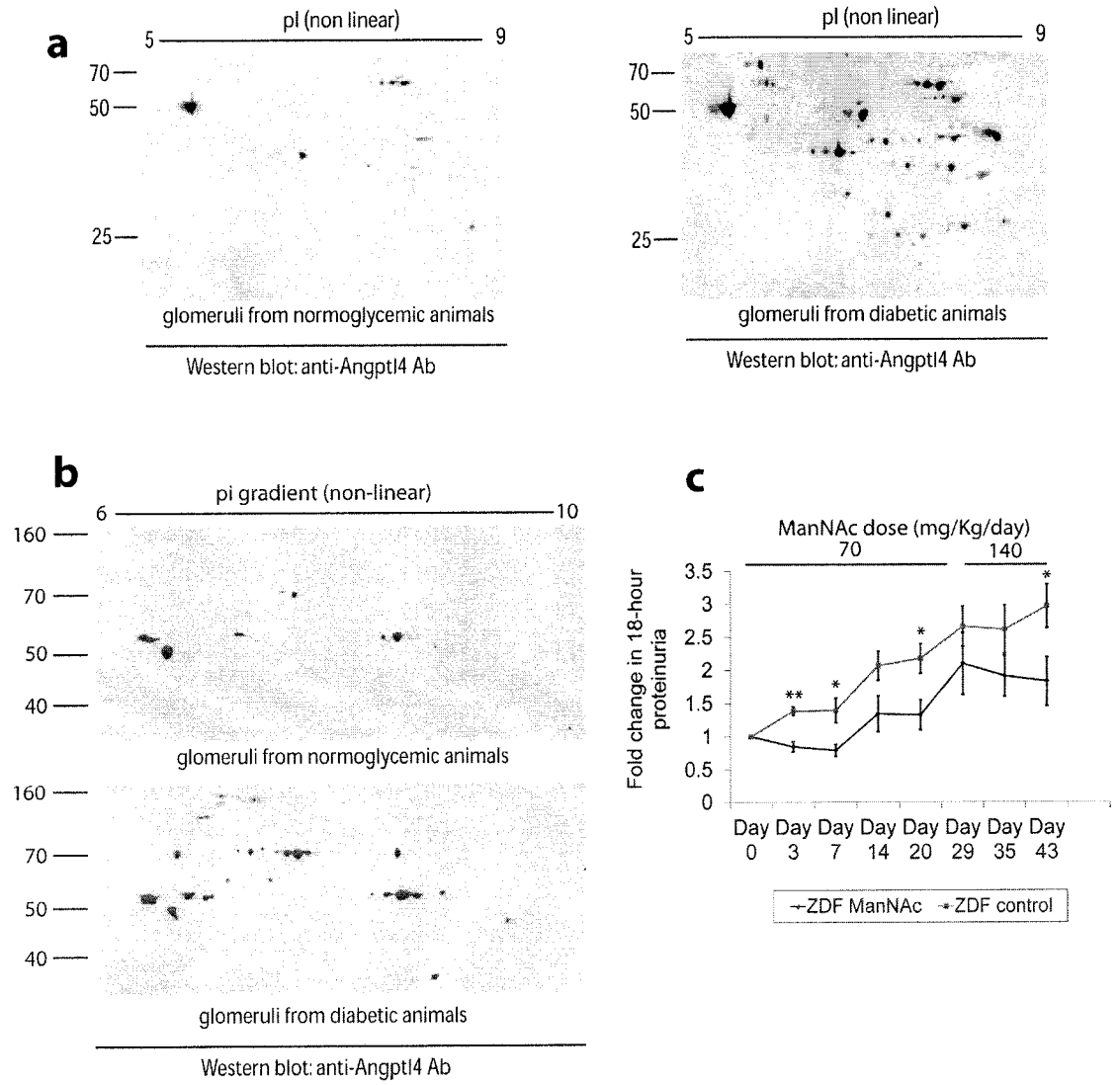

a
pI (non linear)
5
9
70
50
25
glomeruli from normoglycemic animals
Western blot: anti-Angptl4 Ab
pI (non linear)
5
9
70
50
25
glomeruli from diabetic animals
Western blot: anti-Angptl4 Ab
b
pi gradient (non-linear)
6
10
160
70
50
40
glomeruli from normoglycemic animals
160
70
50
40
glomeruli from diabetic animals
Western blot: anti-Angptl4 Ab
c
ManNAc dose (mg/Kg/day)
70
140
Fold change in 18-hour proteinuria
3.5
3
2.5
2
1.5
1
0.5
0
**
*
*
*
Day 0
Day 3
Day 7
Day 14
Day 20
Day 29
Day 35
Day 43
ZDF ManNAc
ZDF control

Figure 8

FIGURE 9

SEQ ID NO: 1

```
MSGAPTAGAA LMLCAATAVL LSAQGGPVQS KSPRFASWDE MNVLAHGLLQ LGQGLREHAE      60
RTRSQLSALE RRLSACGSAC QGTEGSTDLP LAPESRVDPE VLHSLQTQLK AQNSRIQQLF     120
HKVAQQQRHL EKQHLRIQHL QSQFGLLDHK HLDHEVAKPA RRKRLPEMAQ PVDPAHNVSR     180
LHHGGWTVIQ RRHDGSVDFN RPWEAYKAGF GDPHGEFWLG LEKVHSITGD RNSRLAVQLR     240
DWDGNAELLQ FSVHLGGEDT AYSLQLTAPV AGQLGATTVP PSGLSVPFST WDQDHDLRRD     300
KNCAKSLSGG WWFGTCSHSN LNGQYFRSIP QQRQKLKKGI FWKTWRGRYY PLQATTMLIQ     360
PMAAEAAS                                                              368
```

SEQ ID NO: 2

```
ataaaaaccg tcctcgggcg cggcggggag aagccgagct gagcggatcc tcacacgact      60
gtgatccgat tctttccagc ggcttctgca accaagcggg tcttaccccc ggtcctccgc     120
gtctccagtc ctcgcacctg gaaccccaac gtccccgaga gtccccgaat ccccgctccc     180
aggctaccta agaggatgag cggtgctccg acggccgggg cagccctgat gctctgcgcc     240
gccaccgccg tgctactgag cgctcagggc ggacccgtgc agtccaagtc gccgcgcttt     300
gcgtcctggg acgagatgaa tgtcctggcg cacggactcc tgcagctcgg ccaggggctg     360
cgcgaacacg cggagcgcac ccgcagtcag ctgagcgcgc tggagcggcg cctgagcgcg     420
tgcgggtccg cctgtcaggg aaccgagggg tccaccgacc tcccgttagc ccctgagagc     480
cgggtggacc ctgaggtcct tcacagcctg cagacacaac tcaaggctca gaacagcagg     540
atccagcaac tcttccacaa ggtggcccag cagcagcggc acctggagaa gcagcacctg     600
cgaattcagc atctgcaaag ccagtttggc ctcctggacc acaagcacct agaccatgag     660
gtggccaagc ctgcccgaag aaagaggctg cccgagatgg cccagccagt tgacccggct     720
cacaatgtca gccgcctgca ccggctgccc agggattgcc aggagctgtt ccaggttggg     780
gagaggcaga gtggactatt tgaaatccag cctcaggggt ctccgccatt tttggtgaac     840
tgcaagatga cctcagatgg aggctggaca gtaattcaga ggcgccacga tggctcagtg     900
gacttcaacc ggccctggga agcctacaag gcggggtttg gggatcccca cggcgagttc     960
tggctgggtc tggagaaggt gcatagcatc acgggggacc gcaacagccg cctggccgtg    1020
cagctgcggg actgggatgg caacgccgag ttgctgcagt tctccgtgca cctgggtggc    1080
gaggacacgg cctatagcct gcagctcact gcacccgtgg ccggccagct gggcgccacc    1140
accgtcccac ccagcggcct ctccgtaccc ttctccactt gggaccagga tcacgacctc    1200
cgcagggaca agaactgcgc caagagcctc tctggaggct ggtggtttgg cacctgcagc    1260
cattccaacc tcaacggcca gtacttccgc tccatcccac agcagcggca gaagcttaag    1320
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gcccacgaaa gacggtgact cttggctctg cccgaggatg tggccgttcc ctgcctgggc    1500
aggggctcca aggaggggcc atctggaaac ttgtggacag agaagaagac cacgactgga    1560
gaagcccccc ttctgagtgc agggggggctg catgcgttgc ctcctgagat cgaggctgca    1620
ggatatgctc agactctaga ggcgtggacc aaggggcatg gagcttcact ccttgctggc    1680
cagggagttg gggactcaga gggaccactt ggggccagcc agactggcct caatggcgga    1740
ctcagtcaca ttgactgacg gggaccaggg cttgtgtggg tcgagagcgc cctcatggtg    1800
ctggtgctgt tgtgtgtagg tcccctgggg acacaagcag gcgccaatgg tatctgggcg    1860
gagctcacag agttcttgga ataaaagcaa cctcagaaca cttaaaaaaa aaaaaaaaaa    1920
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa                  1967
```

SEQ ID NO: 3

```
MSGAPTAGAA LMLCAATAVL LSAQGGPVQS KSPRFASWDE MNVLAHGLLQ LGQGLREHAE      60
RTRSQLSALE RRLSACGSAC QGTEGSTDLP LAPESRVDPE VLHSLQTQLK AQNSRIQQLF     120
HKVAQQQRHL EKQHLRIQHL QSQFGLLDHK HLDHEVAKPA RRKRLPEMAQ PVDPAHNVSR     180
LHHGGWTVIQ RRHDGSVDFN RPWEAYKAGF GDPHGEFWLG LEKVHSITGD RNSRLAVQLR     240
DWDGNAELLQ FSVHLGGEDT AYSLQLTAPV AGQLGATTVP PSGLSVPFST WDQDHDLRRD     300
KNCAKSLSGG WWFGTCSHSN LNGQYFRSIP QQRQKLKKGI FWKTWRGRYY PLQATTMLIQ     360
PMAAEAAS                                                              368
```

SEQ ID NO: 4

```
ataaaaaccg tcctcgggcg cggcggggag aagccgagct gagcggatcc tcacacgact      60
gtgatccgat tctttccagc ggcttctgca accaagcggg tcttaccccc ggtcctccgc     120
gtctccagtc ctcgcacctg gaaccccaac gtccccgaga gtccccgaat ccccgctccc     180
aggctaccta agaggatgag cggtgctccg acggccgggg cagccctgat gctctgcgcc     240
gccaccgccg tgctactgag cgctcagggc ggacccgtgc agtccaagtc gccgcgcttt     300
gcgtcctggg acgagatgaa tgtcctggcg cacggactcc tgcagctcgg ccaggggctg     360
cgcgaacacg cggagcgcac ccgcagtcag ctgagcgcgc tggagcggcg cctgagcgcg     420
tgcgggtccg cctgtcaggg aaccgagggg tccaccgacc tcccgttagc ccctgagagc     480
cgggtggacc ctgaggtcct tcacagcctg cagacacaac tcaaggctca gaacagcagg     540
atccagcaac tcttccacaa ggtggcccag cagcagcggc acctggagaa gcagcacctg     600
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cacaatgtca gccgcctgca ccatggaggc tggacagtaa ttcagaggcg ccacgatggc     780
tcagtggact tcaaccggcc ctgggaagcc tacaaggcgg ggtttgggga tccccacggc     840
```

```
gagttctggc tgggtctgga gaaggtgcat agcatcacgg gggaccgcaa cagccgcctg       900
gccgtgcagc tgcgggactg ggatggcaac gccgagttgc tgcagttctc cgtgcacctg       960
ggtggcgagg acacggccta tagcctgcag ctcactgcac ccgtggccgg ccagctgggc      1020
gccaccaccg tcccacccag cggcctctcc gtacccttct ccacttggga ccaggatcac      1080
gacctccgca gggacaagaa ctgcgccaag agcctctctg gaggctggtg gtttggcacc      1140
tgcagccatt ccaacctcaa cggccagtac ttccgctcca tcccacagca gcggcagaag      1200
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atggtgctgg tgctgttgtg tgtaggtccc ctggggacac aagcaggcgc caatggtatc      1740
tgggcggagc tcacagagtt cttggaataa aagcaacctc agaacactta aaaaaaaaaa      1800
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa             1853
```

SEQ ID NO: 5

```
MRCAPTAGAA LVLCAATAGL LSAQGRPAQP EPPRFASWDE MNLLAHGLLQ LGHGLREHVE       60
RTRGQLGALE RRMAACGNAC QGPKGTDPKD RVPEGQAPET LQSLQTQLKA QNSKIQQLFQ      120
KVAQQQRYLS KQNLRIQNLQ SQIDLLTPTH LDNGVDKTSR GKRLPKMAQL IGLTPNATRL      180
HRPPRDCQEL FQEGERHSGL FQIQPLGSPP FLVNCEMTSD GGWTVIQRRL NGSVDFNQSW      240
EAYKDGFGDP QGEFWLGLEK MHSITGDRGS QLAVQLQDWD GNAKLLQFPI HLGGEDTAYS      300
LQLTEPTANE LGATNVSPNG LSLPFSTWDQ DHDLRGDLNC AKSLSGGWWF GTCSHSNLNG      360
QYFHSIPRQR QQRKKGIFWK TWKGRYYPLQ ATTLLIQPME ATAAS                       405
```

SEQ ID NO: 6

```
atgcgctgcg ctccgaccgc aggcgctgct ctagtgctat gcgcagctac tgcggggctg        60
ctgagcgcgc aagggcgccc tgcacagccg gagccgccgc gcttcgcatc ctgggatgaa       120
atgaacttgc tggctcacgg gctgctgcag ctcggtcacg ggctgcggga acacgtggag       180
cgcacccgtg gacagctggg cgcgctggaa cgccgcatgg ctgcctgcgg taacgcttgt       240
caggggccca aggggacaga cccgaaggat agagtccccg aaggccaggc tcctgagact       300
ctgcagagtt tacagactca actcaaggct cagaacagca agatccagca actgttccag       360
aaggtagccc agcagcagag atacctatca aagcagaatc tgagaataca gaatcttcag       420
agccagattg acctcttgac ccccacacac ctagacaatg gggtagacaa gacttcgagg       480
ggaaagaggc ttcccaagat ggcccagctc attggcttga ctcccaacgc cacccgctta       540
cacaggcctc cccgggactg ccaggaactc tttcaagaag gggagcggca cagtggactt       600
ttccagatcc agcctctggg atctccacca tttttggtca actgtgagat gacttcagat       660
ggaggctgga cggtgattca gagacgcctg aacggctctg tggacttcaa tcagtcttgg       720
gaagcctaca aagatggctt cggagatccc caaggcgagt tctggctggg cctagagaag       780
atgcacagca tcacagggga ccgaggaagc cagttggctg tgcagctcca ggactgggat       840
ggcaatgcca aattgctcca atttcctatc catttggggg gtgaggacac agcctacagc       900
ctgcagctca ccgagcccac ggccaatgag ctgggtgcca ccaatgtttc ccccaatggc       960
ctttccctgc ccttctctac ctgggaccaa gaccacgacc tccgagggga ccttaactgt      1020
gccaagagcc tctctggtgg ctggtggttt ggcacctgca gccattccaa tctaaatgga      1080
caatacttcc actctattcc acggcaacgg cagcagcgta aaaaggggat cttctggaaa      1140
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gctacagcag cctcttag                                                    1218
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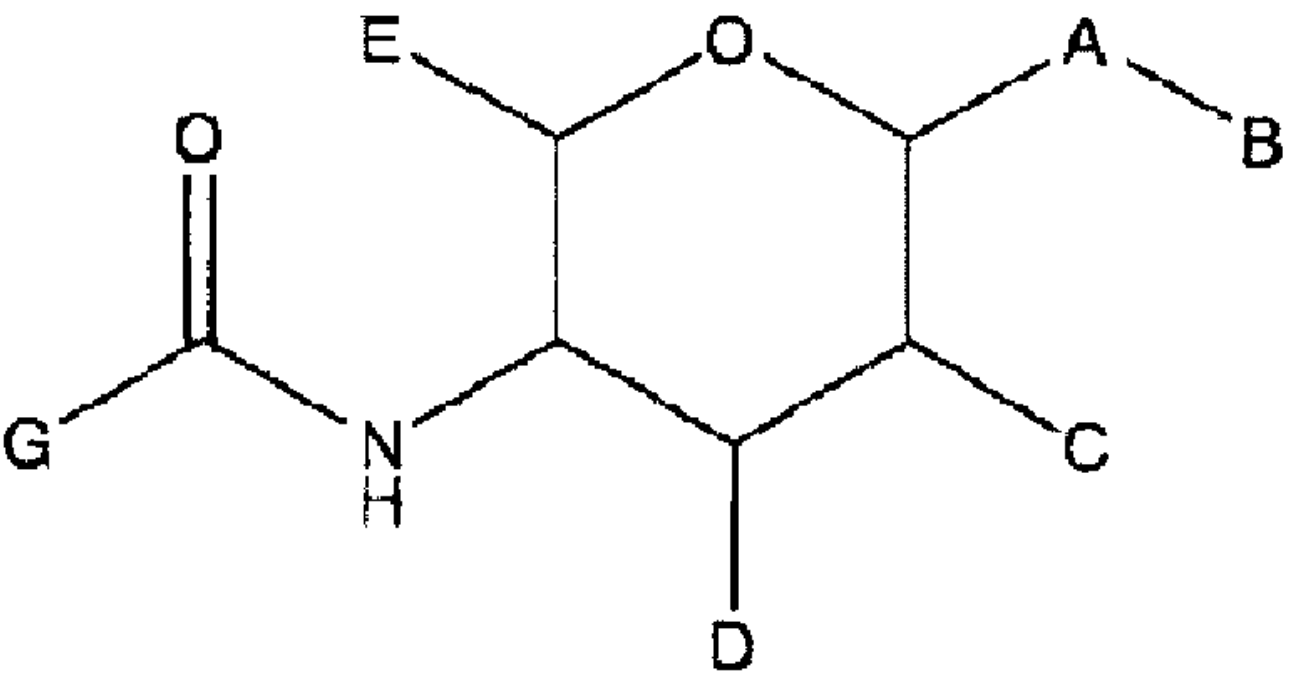
SEQ ID NO: 7

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RTRGQLGALE RRMAACGNAC QGPKGKDAPF KDSEDRVPEG QTPETLQSLQ TQLKAQNSKI      120
QQLFQKVAQQ QRYLSKQNLR IQNLQSQIDL LAPTHLDNGV DKTSRGKRLP KMTQLIGLTP      180
NATHLHRPPR DCQELFQEGE RHSGLFQIQP LGSPPFLVNC EMTSDGGWTV IQRRLNGSVD      240
FNQSWEAYKD GFGDPQGEFW LGLEKMHSIT GNRGSQLAVQ LQDWDGNAKL LQFPIHLGGE      300
DTAYSLQLTE PTANELGATN VSPNGLSLPF STWDQDHDLR GDLNCAKSLS GGWWFGTCSH      360
SNLNGQYFHS IPRQRQERKK GIFWKTWKGR YYPLQATTLL IQPMEATAAS                  410
```

SEQ ID NO: 8

```
acgggctcca gatcttcttc tgcaccagag caagtctaag tctgagccgg ctcccccaga        60
actccagctg ctgggtcttg aactcctgcg ttccggagtc ctagcgttgc tgcacccaag       120
gccaccccca gaatcatgcg ctgcgctccg acagcaggcg ctgccctggt gctatgcgcg       180
gctactgcgg ggcttttgag cgcgcaaggg cgccctgcac agccagagcc accgcgcttt       240
gcatcctggg acgagatgaa cttgctggct cacgggctgc tacagctcgg ccatgggctg       300
cgcgaacacg tggagcgcac ccgtgggcag ctgggcgcgc tggagcgccg catggctgcc       360
tgtggtaacg cttgtcaggg gcccaaggga aaagatgcac ccttcaaaga ctccgaggat       420
agagtccctg aaggccagac tcctgagact ctgcagagtt tgcagactca gctcaaggct       480
caaaacagca agatccagca attgttccag aaggtggccc agcagcagag atacctatca       540
aagcagaatc tgagaataca gaatcttcag agccagatag acctcttggc ccccacgcac       600
```

ctagacaatg gagtagacaa gacttcgagg ggaaagaggc ttcccaagat gacccagctc 660
attggcttga ctcccaacgc cacccactta cacaggccgc cccgggactg ccaggaactc 720
ttccaagaag gggagaggca cagtggactt ttccagatcc agcctctggg gtctccacca 780
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catttggggg gtgaggacac agcctacagc ctgcagctca ctgagcccac ggccaatgag 1080
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gaccatgacc tccgtgggga ccttaactgt gccaagagcc tctctggtgg ctggtggttt 1200
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ccgtaactgg agagactgga ggaccccttt tccgtgttgg ggtctgcaag cattgttgtc 1560
tgaaacagtc agagcaacag gaaacaaatg gcccagatcc agaaaacatg ggctcgaggg 1620
gcactgaata tcacttctcg cctaccagag aagttgggga tgcagaggga ccactacagt 1680
ccaactagct gggcccttaa tggcggactc agtcatattg actgactgga gacagggtgc 1740
caggagccct ggatacactc atggtgctgt tgtaggtgct gtggatgcac aggtgctaac 1800
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aaaaaaaaa 1869


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