

Treatment Of Kidney Diseases With Angiopoietin Like 3 (ANGPTL3) Inhibitors

Reference To Sequence Listing

This application includes a Sequence Listing filed electronically as an XML file named
5 381203629SEQ, created on November 28, 2022, with a size of 1,673 kilobytes. The Sequence
Listing is incorporated herein by reference.

Field

The present disclosure relates generally to the treatment of subjects having a kidney
10 disease or at risk of developing a kidney disease with a Angiopoietin Like 3 (ANGPTL3) inhibitor,
and methods of identifying subjects having an increased risk of developing a kidney disease.

Background

In the United States, based on data from the 1999-2006 National Health and Nutrition
15 Examination Survey (NHANES) study, an estimated 11.1 percent (22.4 million) of adults aged 20
or older have chronic kidney disease (CKD) stages 1-3. An additional 0.8 million U.S. adults aged
20 or older have CKD stage 4, and more than 0.3 million have stage 5 CKD and receive
hemodialysis. Analyses of NHANES data between 1988-1994 and 1999-2004 suggest that the
prevalence of CKD is rising for every CKD stage, but with a particular increase in the prevalence
20 of individuals classified with CKD stage 3. The number of patients with stage 5 CKD requiring
dialysis also has increased. It has been estimated that more than 700,000 individuals will have
End Stage Renal Disease (ESRD) by 2015. Although CKD can be caused by primary kidney
disease (e.g., glomerular diseases, tubulointerstitial diseases, obstruction, and polycystic kidney
disease), in the vast majority of patients with CKD, the kidney damage is associated with other
25 medical conditions such as diabetes and hypertension. In 2008, excluding those with ESRD, 48
percent of Medicare patients with CKD had diabetes, 91 percent had hypertension, and 46
percent had atherosclerotic heart disease. Other risk factors for CKD include age, obesity,
family history, and ethnicity. CKD has been associated with numerous adverse health
outcomes.

30 A Glomerular Filtration Rate (GFR) of 90 mL/min or higher (Stage 1) is normal in most
healthy people. Usually few symptoms are present at this stage of CKD. A GFR of 60-89 mL/min
(Stage 2) may for some patients, such as the elderly or infants, be normal if no kidney damage

is present. A GFR between 60-89 mL/min for three months or longer along with kidney damage is a sign of early CKD. Usually few symptoms are present at this stage. A GFR between 30-59 mL/min (Stage 3) for a patient is indicative of moderate CKD, and are more likely to develop anemia, early bone disease or high blood pressure, and may desire to see a nephrologist. A GFR
5 between 15-29 mL/min (Stage 4) indicates that the patient has severe CKD, and will likely need dialysis or a kidney transplant in the future. A GFR of 15 mL/min or less (Stage 5) indicates that the patient has chronic CKD, and have ESRD. The kidneys have lost almost all ability to function effectively at this stage. They will need dialysis or a kidney transplant to live.

The ANGPTL3 gene encodes a member of a family of secreted proteins that function in
10 angiogenesis. The encoded protein, which is expressed predominantly in the liver, is further processed into an N-terminal coiled-coil domain-containing chain and a C-terminal fibrinogen chain. The N-terminal chain is important for lipid metabolism, while the C-terminal chain may be involved in angiogenesis. Mutations in this gene cause familial hypobetalipoproteinemia type 2.

Summary

The present disclosure provides methods of treating a subject having a kidney disease or at risk of developing a kidney disease, the methods comprising administering an ANGPTL3 inhibitor to the subject, wherein the kidney disease is not nephrotic syndrome.

20 The present disclosure also provides methods of treating a subject having a kidney disease or at risk of developing a kidney disease by administering a kidney disease therapeutic agent, the methods comprising: determining whether the subject has an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule by: obtaining or having obtained a biological sample from the subject; and performing or having performed a sequence analysis on
25 the biological sample to determine if the subject has a genotype comprising the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule; and administering or continuing to administer the kidney disease therapeutic agent in a standard dosage amount to a subject that is ANGPTL3 reference, and/or administering an ANGPTL3 inhibitor to the subject; administering or continuing to administer the kidney disease therapeutic agent in an amount
30 that is the same as or less than a standard dosage amount to a subject that is heterozygous for the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, and/or administering an ANGPTL3 inhibitor to the subject; or administering or continuing to administer

the kidney disease therapeutic agent in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule; wherein the presence of a genotype having the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule indicates the subject has a decreased risk of developing a kidney disease; and wherein the kidney disease is not nephrotic syndrome.

Disclosed herein is a method of identifying a subject having an increased risk of developing a kidney disease, the method comprising: determining or having determined the presence or absence of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule in a biological sample obtained from the subject; wherein: when the subject is ANGPTL3 reference, then the subject has an increased risk of developing a kidney disease; and when the subject is heterozygous or homozygous for the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, then the subject has a decreased risk of developing a kidney disease; and wherein the kidney disease is not nephrotic syndrome.

The present disclosure also provides kidney disease therapeutic agent for use in the treatment and/or prevention of a kidney disease in a subject having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome.

The present disclosure provides the use of a kidney disease therapeutic agent in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome, and wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region.

The present disclosure also provides ANGPTL3 inhibitors for use in the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome.

Disclosed herein is the use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic

syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to an ANGPTL3 nucleic acid molecule.

5 Disclosed herein is the use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid
10 molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises a Cas protein and guide RNA (gRNA) that hybridizes to a gRNA recognition sequence within an ANGPTL3 genomic nucleic acid molecule.

Disclosed herein is the use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a
15 subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises a small molecule.

20 Disclosed herein is the use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid
25 molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises an antibody.

Brief Description Of The Drawings

The accompanying figures, which are incorporated in and constitute a part of this
30 specification, illustrate several features of the present disclosure.

Figure 1 shows associations of circulating levels of ANGPTL3 measured at baseline samples with risk of newly-onset chronic kidney disease (n=977/38,043) and the observed risk

of newly-onset chronic kidney disease among the quartiles of plasma ANGPTL3 measured via Olink® Explore 1536 assay; analyses were performed among individuals without prior history of chronic kidney disease at baseline; the adjusted hazard ratios and corresponding confidence intervals were estimated using Cox regression, stratified by sex and study center, adjusted for age-at-baseline, systolic blood pressure, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, smoking status, alcohol consumption, history of diabetes, and eGFR_{creatinine}; abbreviations: HR, hazard ratios, CI, confidence interval, SD, standard deviation, Q1-Q5: the 1st quartile, ..., the fifth quartile.

Figure 2 (Panel A) is a graph depicting the results of urinary albumin normalized to urinary creatinine in wildtype lean (Lean) and uninephrectomized *Db/Db* C57BLKS (*Db/Db*) mice; Figure 2 (Panel B) is a graph depicting the results of an ELISA analysis of serum ANGPTL3 in wildtype lean (Lean) and uninephrectomized *Db/Db* C57BLKS (*Db/Db*) mice. **, unpaired t-test $p < 0.01$.

Figure 3 (Panel A) is a graph depicting the results of urinary albumin normalized to urinary creatinine in sham-operated (Sham) and 5/6th nephrectomy remnant kidney model (RKM) mice; Figure 3 (Panel B) is a graph depicting the results of an ELISA analysis of serum ANGPTL3 in in sham-operated (Sham) and 5/6th nephrectomy remnant kidney model (RKM) mice. *, unpaired t-test $p < 0.05$; *** unpaired t-test $p < 0.0005$

Description

Various terms relating to aspects of the present disclosure are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art, unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not specifically state in the claims or descriptions that the steps are to be limited to a specific order, it is in no way intended that an order be inferred, in any respect. This holds for any possible non-expressed basis for interpretation, including matters of logic with respect to arrangement of steps or operational flow, plain meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

As used herein, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

As used herein, the term “about” means that the recited numerical value is approximate and small variations would not significantly affect the practice of the disclosed
5 embodiments. Where a numerical value is used, unless indicated otherwise by the context, the term “about” means the numerical value can vary by $\pm 10\%$ and remain within the scope of the disclosed embodiments.

As used herein, the term “comprising” may be replaced with “consisting” or “consisting essentially of” in particular embodiments as desired.

10 As used herein, the term “isolated”, in regard to a nucleic acid molecule or a polypeptide, means that the nucleic acid molecule or polypeptide is in a condition other than its native environment, such as apart from blood and/or animal tissue. In some embodiments, an isolated nucleic acid molecule or polypeptide is substantially free of other nucleic acid molecules or other polypeptides, particularly other nucleic acid molecules or polypeptides of
15 animal origin. In some embodiments, the nucleic acid molecule or polypeptide can be in a highly purified form, i.e., greater than 95% pure or greater than 99% pure. When used in this context, the term “isolated” does not exclude the presence of the same nucleic acid molecule or polypeptide in alternative physical forms, such as dimers or Alternately phosphorylated or derivatized forms.

20 As used herein, the terms “nucleic acid”, “nucleic acid molecule”, “nucleic acid sequence”, “polynucleotide”, or “oligonucleotide” can comprise a polymeric form of nucleotides of any length, can comprise DNA and/or RNA, and can be single-stranded, double-stranded, or multiple stranded. One strand of a nucleic acid also refers to its complement.

As used herein, the term “subject” includes any animal, including mammals. Mammals
25 include, but are not limited to, farm animals (such as, for example, horse, cow, pig), companion animals (such as, for example, dog, cat), laboratory animals (such as, for example, mouse, rat, rabbits), and non-human primates. In some embodiments, the subject is a human. In some embodiments, the human is a patient under the care of a physician.

It has been observed in accordance with the present disclosure that ANGPTL3
30 predicted loss-of-function or missense variant nucleic acid molecules (whether these variants are homozygous or heterozygous in a particular subject) associate with a decreased risk of developing a kidney disease. It is believed that ANGPTL3 predicted loss-of-function coding

variant nucleic acid molecules have not been associated with kidney disease. Therefore, subjects that are ANGPTL3 reference or heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule may be treated with an ANGPTL3 inhibitor such that a kidney disease is inhibited or prevented, the symptoms thereof are reduced or prevented, and/or development of symptoms is repressed or prevented. It is also believed that such subjects having a kidney disease may further be treated with a kidney disease therapeutic agent.

For purposes of the present disclosure, any particular subject, such as a human, can be categorized as having one of three ANGPTL3 genotypes: i) ANGPTL3 reference; ii) heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule; or iii) homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. A subject is ANGPTL3 reference when the subject does not have a copy of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. A subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule when the subject has a single copy of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. An ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is any nucleic acid molecule (such as, a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule) encoding a variant ANGPTL3 polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in a coding region. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule does not comprise a variation in a non-coding region, except for splice acceptor regions (two bases before the start of any exon except the first). A subject who has an ANGPTL3 polypeptide having a partial loss-of-function (or predicted partial loss-of-function) is hypomorphic for ANGPTL3. A subject is homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule when the subject has two copies (same or different) of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule.

For subjects that are genotyped or determined to be ANGPTL3 reference, such subjects have an increased risk of developing a kidney disease. For subjects that are genotyped or determined to be either ANGPTL3 reference or heterozygous for an ANGPTL3 predicted loss-

of-function or missense variant nucleic acid molecule, such subjects or subjects can be treated with an ANGPTL3 inhibitor.

In any of the embodiments described herein, the subject in whom a kidney disease is treated or prevented by administering the ANGPTL3 inhibitor can be anyone at risk for developing a kidney disease including, but not limited to, subjects with a genetic predisposition for developing a kidney disease. Additional risk factors include, but are not limited to, diabetes, hypertension, obesity, excessive salt intake, age, smoking, excessive alcohol consumption, heavy metal exposure, hyperlipidemia, and the presence of autoimmune diseases. In addition, in some embodiments, any subject can be at risk of developing a kidney disease. In some embodiments, administering an ANGPTL3 inhibitor may be carried out to prevent development of an additional kidney disease in a subject who has already had a kidney disease.

In any of the embodiments described herein, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can be any nucleic acid molecule (such as, for example, genomic nucleic acid molecule, mRNA molecule, or cDNA molecule) encoding an ANGPTL3 variant polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is any nucleic acid molecule (such as, a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule) resulting in decreased or aberrant expression of ANGPTL3 mRNA or polypeptide. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is associated with a reduced *in vitro* response to ANGPTL3 ligands compared with reference ANGPTL3. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is an ANGPTL3 variant nucleic acid molecule that results or is predicted to result in a premature truncation of an ANGPTL3 polypeptide compared to the human reference genome sequence. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is a variant that is predicted to be damaging to the protein function (and hence, in this case, protective to the human) by *in vitro* prediction algorithms such as Polyphen, SIFT, or similar algorithms. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is a variant that causes or is predicted to cause a nonsynonymous amino-acid substitution in an ANGPTL3 nucleic acid molecule and whose allele frequency is less than 1/100 alleles in the population from which the subject is selected. In some embodiments,

the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is any rare missense variant (allele frequency < 0.1%; or 1 in 1,000 alleles), or any splice-site, stop-gain, start-loss, stop-loss, frameshift, or in-frame indel, or other frameshift ANGPTL3 variant.

In any of the embodiments described herein, the ANGPTL3 predicted loss-of-function polypeptide can be any ANGPTL3 polypeptide having a partial loss-of-function, a complete loss-of-function, a predicted partial loss-of-function, or a predicted complete loss-of-function.

In any of the embodiments described herein, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can include variations at positions of chromosome 1 using the nucleotide sequence of the ANGPTL3 reference genomic nucleic acid molecule (see, ENST00000371129 annotated in the in the Ensembl database (URL: world wide web at ["//useast.ensembl.org/index.html"](http://useast.ensembl.org/index.html))) as a reference sequence. The sequence provided in ENST00000371129 for the ANGPTL3 genomic nucleic acid molecule is only an exemplary sequence. Other sequences for the ANGPTL3 genomic nucleic acid molecule are also possible. Exemplary ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules include, but are not limited to those recited in Table 4.

Any one or more (i.e., any combination) of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules described herein can be used within any of the methods described herein to determine whether a subject has an increased or decreased risk of developing a kidney disease. The combinations of particular variants can form a mask used for statistical analysis of the particular correlation of ANGPTL3 and an increased or decreased risk of developing a kidney disease. In some embodiments, the mask used for statistical analysis of the particular correlation of ANGPTL3 and an increased or decreased risk of developing a kidney disease can exclude any one or more of these ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules described herein.

In any of the embodiments described herein, the subject can have a kidney disease. In any of the embodiments described herein, the subject can be at risk of developing a kidney disease. In any of the embodiments described herein, the kidney disease is chronic kidney disease, a kidney stone, chronic glomerulonephritis, nephronophthisis, chronic interstitial nephritis, and/or nephrosclerosis. In some embodiments, the kidney disease is chronic kidney disease. In some embodiments, the kidney disease is a kidney stone. In some embodiments, the kidney disease is chronic glomerulonephritis. In some embodiments, the kidney disease is nephronophthisis. In some embodiments, the kidney disease is chronic interstitial nephritis. In

some embodiments, the kidney disease is nephrosclerosis. In any of the embodiments described herein, the kidney disease is not nephrotic syndrome.

Other kidney disease include, but are not limited to, acquired cystic disease, acute (postinfectious) glomerulonephritis, acute infectious interstitial nephritis, acute interstitial
5 nephritis, acute pyelonephritis, acute renal failure, acute transplant failure, acute tubular necrosis, adult polycystic kidney disease, AL amyloid, analgesic nephrosis, ANCA-associated vasculitis, anti-glomerular basement membrane disease (Goodpasture's Syndrome), antibody-mediated kidney graft rejection, asymptomatic hematuria, asymptomatic proteinuria, atypical hemolytic uremic syndrome, autosomal dominant polycystic kidney disease, autosomal
10 recessive polycystic kidney disease, BK virus-associated nephropathy, Bence Jones cast nephrosis, benign familial hematuria, benign nephrosclerosis and atheromatous embolization, bilateral cortical necrosis, C3 glomerulonephritis, cardiac surgery associated acute kidney injury, chronic allograft nephropathy, chronic glomerulonephritis, chronic interstitial nephritis, chronic pyelonephritis, chronic renal failure, chronic transplant failure, circulating immune complex
15 nephritis, contrast-induced nephropathy, crescentic glomerulonephritis, cryoglobulinemia, cystic renal dysplasia, delayed graft function, dense deposit disease, diabetic glomerulosclerosis, diabetic nephropathy, dialysis cystic disease, drug induced (allergic) acute interstitial nephritis, ectopic kidney, eosinophilic granulomatosis with polyangiitis, Fabry's disease, familial juvenile nephronophthisis-medullary cystic disease complex, focal segmental
20 glomerulosclerosis (segmental hyalinosis), glomerulocystic disease, glomerulonephritis, glomerulonephritis associated with bacterial endocarditis, glomerulosclerosis, granulomatosis with polyangiitis, hemolytic-uremic syndrome, Henoch-Schonlein purpura, hepatitis-associated glomerulonephritis, hereditary nephritis (Alport syndrome), Human Immunodeficiency Virus-associated nephropathy, horseshoe kidney, hydronephrosis, hyperoxaluria, hypertensive
25 nephropathy, IgA nephropathy, infantile polycystic kidney disease, ischemic acute tubular necrosis, light-chain deposit disease, lupus nephritis, malignant nephrosclerosis, medullary cystic disease, membranoproliferative (mesangiocapillary) glomerulonephritis, membranous glomerulonephritis, membranous nephropathy, mesangial proliferative glomerulonephritis (includes Berger's Disease), microscopic polyangiitis, minimal change glomerular disease,
30 nephritic syndrome, nephroblastoma (Wilms tumor), nephronophthisis (medullary cystic disease complex), pigment nephropathy, plasma cell dyscrasias (monoclonal immunoglobulin-induced renal damage), polyarteritis nodosa, polycystic kidney disease, proteinuria,

pyelonephritis, rapidly progressive (crescentic) glomerulonephritis, renal agenesis, renal amyloidosis, renal cell carcinoma, renal dysgenesis, renal dysplasia, renal hypoplasia, renal infection, renal osteodystrophy, renal stones (urolithiasis), renal tubular acidosis, renal vasculitis, renovascular hypertension, scleroderma (progressive systemic sclerosis), secondary
5 acquired glomerulonephritis, sepsis-associated acute kidney injury, simple renal cysts, systemic lupus erythematosus, T-cell-mediated kidney graft rejection, thin basement membrane nephropathy, thrombotic microangiopathy, thrombotic thrombocytopenic purpura, toxic acute tubular necrosis, tubular defects, tubulointerstitial disease in multiple myeloma, urate nephropathy, urinary obstruction, and vasculitis.

10 Symptoms of chronic kidney disease include, but are not limited to, nausea, vomiting, loss of appetite, fatigue and weakness, sleep problems, changes urination volume, decreased mental sharpness, muscle twitches and cramps, swelling of feet and ankles, persistent itching, chest pain, fluid build-up around the lining of the heart, shortness of breath, fluid build-up in the lungs, and high blood pressure (hypertension) that's difficult to control.

15 Symptoms of a kidney stone include, but are not limited to, severe, sharp pain in the side and back, below the ribs, pain that radiates to the lower abdomen and groin, pain that comes in waves and fluctuates in intensity, pain or burning sensation while urinating, pink, red or brown urine, cloudy or foul-smelling urine, a persistent need to urinate, urinating more often than usual or urinating in small amounts, nausea and vomiting, and fever and chills if an
20 infection is present.

Symptoms of chronic glomerulonephritis include, but are not limited to, pink or cola-colored urine from red blood cells in your urine (hematuria), foamy urine due to excess protein (proteinuria), high blood pressure (hypertension), and fluid retention (edema) with swelling evident in the face, hands, feet and abdomen.

25 Symptoms of nephronophthisis include, but are not limited to, increased urine production (polyuria), excessive thirst (polydipsia), general weakness, and extreme tiredness (fatigue).

Symptoms of chronic interstitial nephritis include, but are not limited to, blood in the urine, fever, increased or decreased urine output, mental status changes (drowsiness,
30 confusion, coma), nausea, vomiting, rash, swelling of any area of body, and weight gain (from retaining fluid).

Symptoms of nephrosclerosis include, but are not limited to, impaired vision, blood in the urine, loss of weight, and the accumulation of urea and other nitrogenous waste products in the blood, a condition known as uremia.

The present disclosure provides methods of treating a subject having a kidney disease
5 or at risk of developing a kidney disease, the methods comprising administering an ANGPTL3 inhibitor to the subject, wherein the kidney disease is not nephrotic syndrome.

In some embodiments, the ANGPTL3 inhibitor comprises an inhibitory nucleic acid molecule. Examples of inhibitory nucleic acid molecules include, but are not limited to, antisense nucleic acid molecules, small interfering RNAs (siRNAs), and short hairpin RNAs
10 (shRNAs). Such inhibitory nucleic acid molecules can be designed to target any region of an ANGPTL3 nucleic acid molecule. In some embodiments, the antisense RNA, siRNA, or shRNA hybridizes to a sequence within an ANGPTL3 genomic nucleic acid molecule or mRNA molecule and decreases expression of the ANGPTL3 polypeptide in a cell in the subject. In some
15 embodiments, the ANGPTL3 inhibitor comprises an antisense molecule that hybridizes to an ANGPTL3 genomic nucleic acid molecule or mRNA molecule and decreases expression of the ANGPTL3 polypeptide in a cell in the subject. In some embodiments, the ANGPTL3 inhibitor comprises an siRNA that hybridizes to an ANGPTL3 genomic nucleic acid molecule or mRNA molecule and decreases expression of the ANGPTL3 polypeptide in a cell in the subject. In some
20 embodiments, the ANGPTL3 inhibitor comprises an shRNA that hybridizes to an ANGPTL3 genomic nucleic acid molecule or mRNA molecule and decreases expression of the ANGPTL3 polypeptide in a cell in the subject.

In some embodiments, the antisense nucleic acid molecules comprise or consist of any of the nucleotide sequences represented by SEQ ID NOs: 179-503. In some embodiments, the siRNA molecules comprise or consist of any of the nucleotide sequences (sense and antisense
25 strands presented one after the other) represented by SEQ ID NOs: 504-1367 (e.g., the sense strand is, for example, SEQ ID NO: 504 and the corresponding antisense strand is SEQ ID NO: 505; the sense strand is, for example, SEQ ID NO: 506 and the corresponding antisense strand is SEQ ID NO: 507; etc.).

The inhibitory nucleic acid molecules can comprise RNA, DNA, or both RNA and DNA.
30 The inhibitory nucleic acid molecules can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the inhibitory nucleic acid molecules can be within a vector or as an exogenous donor sequence comprising the inhibitory

nucleic acid molecule and a heterologous nucleic acid sequence. The inhibitory nucleic acid molecules can also be linked or fused to a heterologous label. The label can be directly detectable (such as, for example, fluorophore) or indirectly detectable (such as, for example, hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels, pigments, dyes, chromogens, spin labels, and fluorescent labels. The label can also be, for example, a chemiluminescent substance; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal. The term "label" can also refer to a "tag" or hapten that can bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, biotin can be used as a tag along with an avidin or streptavidin conjugate of horseradish peroxidase (HRP) to bind to the tag, and examined using a calorimetric substrate (such as, for example, tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. Exemplary labels that can be used as tags to facilitate purification include, but are not limited to, myc, HA, FLAG or 3XFLAG, 6XHis or polyhistidine, glutathione-S-transferase (GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

The inhibitory nucleic acid molecules can comprise, for example, nucleotides or non-natural or modified nucleotides, such as nucleotide analogs or nucleotide substitutes. Such nucleotides include a nucleotide that contains a modified base, sugar, or phosphate group, or that incorporates a non-natural moiety in its structure. Examples of non-natural nucleotides include, but are not limited to, dideoxynucleotides, biotinylated, aminated, deaminated, alkylated, benzylated, and fluorophore-labeled nucleotides.

The inhibitory nucleic acid molecules can also comprise one or more nucleotide analogs or substitutions. A nucleotide analog is a nucleotide which contains a modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety include, but are not limited to, natural and synthetic modifications of A, C, G, and T/U, as well as different purine or pyrimidine bases such as, for example, pseudouridine, uracil-5-yl, hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. Modified bases include, but are not limited to, 5-methylcytosine

(5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (such as, for example, 5-bromo), 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine, 7-methyladenine, 8-azaguanine, 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, and 3-deazaadenine.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety include, but are not limited to, natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include, but are not limited to, the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl, and alkynyl may be substituted or unsubstituted C₁₋₁₀alkyl or C₂₋₁₀alkenyl, and C₂₋₁₀alkynyl. Exemplary 2' sugar modifications also include, but are not limited to, -O[(CH₂)_nO]_mCH₃, -O(CH₂)_nOCH₃, -O(CH₂)_nNH₂, -O(CH₂)_nCH₃, -O(CH₂)_n-ONH₂, and -O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m, independently, are from 1 to about 10. Other modifications at the 2' position include, but are not limited to, C₁₋₁₀alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars can also include those that contain modifications at the bridging ring oxygen, such as CH₂ and S. Nucleotide sugar analogs can also have sugar mimetics, such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include, but are not limited to, those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including

3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. These phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts, and free acid forms are also included. Nucleotide substitutes also include peptide nucleic acids (PNAs).

In some embodiments, the antisense nucleic acid molecules are gapmers, whereby the first one to seven nucleotides at the 5' and 3' ends each have 2'-methoxyethyl (2'-MOE) modifications. In some embodiments, the first five nucleotides at the 5' and 3' ends each have 2'-MOE modifications. In some embodiments, the first one to seven nucleotides at the 5' and 3' ends are RNA nucleotides. In some embodiments, the first five nucleotides at the 5' and 3' ends are RNA nucleotides. In some embodiments, each of the backbone linkages between the nucleotides is a phosphorothioate linkage.

In some embodiments, the siRNA molecules have termini modifications. In some embodiments, the 5' end of the antisense strand is phosphorylated. In some embodiments, 5'-phosphate analogs that cannot be hydrolyzed, such as 5'-(E)-vinyl-phosphonate are used.

In some embodiments, the siRNA molecules have backbone modifications. In some embodiments, the modified phosphodiester groups that link consecutive ribose nucleosides have been shown to enhance the stability and *in vivo* bioavailability of siRNAs. The non-ester groups (-OH, =O) of the phosphodiester linkage can be replaced with sulfur, boron, or acetate to give phosphorothioate, boranophosphate, and phosphonoacetate linkages. In addition, substituting the phosphodiester group with a phosphotriester can facilitate cellular uptake of siRNAs and retention on serum components by eliminating their negative charge. In some embodiments, the siRNA molecules have sugar modifications. In some embodiments, the sugars are deprotonated (reaction catalyzed by exo- and endonucleases) whereby the 2'-hydroxyl can act as a nucleophile and attack the adjacent phosphorous in the phosphodiester bond. Such alternatives include 2'-O-methyl, 2'-O-methoxyethyl, and 2'-fluoro modifications.

In some embodiments, the siRNA molecules have base modifications. In some embodiments, the bases can be substituted with modified bases such as pseudouridine, 5'-methylcytidine, N6-methyladenosine, inosine, and N7-methylguanosine.

In some embodiments, the siRNA molecules are conjugated to lipids. Lipids can be conjugated to the 5' or 3' termini of siRNA to improve their *in vivo* bioavailability by allowing them to associate with serum lipoproteins. Representative lipids include, but are not limited to, cholesterol and vitamin E, and fatty acids, such as palmitate and tocopherol.

5 In some embodiments, a representative siRNA has the following formula:

Sense: mN*mN*/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/
i2FN/*mN*/32FN/

Antisense: /52FN/*/i2FN/*mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/i2FN/mN/
i2FN/mN/i2FN/mN*N*N

10 wherein: "N" is the base; "2F" is a 2'-F modification; "m" is a 2'-O-methyl modification, "I" is an internal base; and "*" is a phosphorothioate backbone linkage.

In any of the embodiments described herein for antisense molecules and siRNA molecules, the molecules can comprise 1, 2, or 3 additional nucleotides at the 5' end, 3' end, or both the 5' end and 3' end. In some embodiments the antisense molecules and siRNA molecules comprise 1, 2, or 3 additional nucleotides at the 5' end. In some embodiments the antisense molecules and siRNA molecules comprise 1, 2, or 3 additional nucleotides at the 3' end. In some embodiments the antisense molecules and siRNA molecules comprise 1, 2, or 3 additional nucleotides at both the 5' end and 3' end.

In any of the embodiments described herein for antisense molecules and siRNA molecules, the molecules can comprise a substantially identical sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% homology to the nucleotide sequences disclosed herein. In some embodiments, the antisense molecules and siRNA molecules have at least 80% homology to the nucleotide sequences disclosed herein. In some embodiments, the antisense molecules and siRNA molecules have at least 85% homology to the nucleotide sequences disclosed herein. In some embodiments, the antisense molecules and siRNA molecules have at least 90% homology to the nucleotide sequences disclosed herein. In some embodiments, the antisense molecules and siRNA molecules have at least 95% homology to the nucleotide sequences disclosed herein. In some embodiments, the antisense molecules and siRNA molecules have at least 98% homology to the nucleotide sequences disclosed herein. In some embodiments, the antisense molecules and siRNA molecules have at least 99% homology to the nucleotide sequences disclosed herein.

In any of the embodiments described herein, the inhibitory nucleic acid molecules may be administered, for example, as one to two hour i.v. infusions or s.c. injections. In any of the embodiments described herein, the inhibitory nucleic acid molecules may be administered at dose levels that range from about 50 mg to about 900 mg, from about 100 mg to about 800 mg, 5 from about 150 mg to about 700 mg, or from about 175 to about 640 mg (2.5 to 9.14 mg/kg; 92.5 to 338 mg/m² – based on an assumption of a body weight of 70 kg and a conversion of mg/kg to mg/m² dose levels based on a mg/kg dose multiplier value of 37 for humans).

In some embodiments, the siRNA molecules comprise or consist of the nucleotide sequences (sense and antisense strands) recited in U.S. Patent No. 10,995,335 and PCT 10 Publication No. WO 2019/055633, which are incorporated herein by reference in their entirety.

In some embodiments, the siRNA molecules comprise or consist of the nucleotide sequences (sense and antisense strands) recited in U.S. Patent No. 10,875,884 and PCT Publication Nos. WO 2015/168589, WO 2015/100394, and WO 2011/085271, which are incorporated herein by reference in their entirety.

15 In some embodiments, the siRNA molecules comprise or consist of the nucleotide sequences (sense and antisense strands) recited in U.S. Patent Nos. 10,570,393 and 10,337,010, and PCT Publication Nos. WO 2016/168286 and WO 2012/177784, which are incorporated herein by reference in their entirety.

The present disclosure also provides vectors comprising any one or more of the 20 inhibitory nucleic acid molecules. In some embodiments, the vectors comprise any one or more of the inhibitory nucleic acid molecules and a heterologous nucleic acid. The vectors can be viral or nonviral vectors capable of transporting a nucleic acid molecule. In some embodiments, the vector is a plasmid or cosmid (such as, for example, a circular double-stranded DNA into which additional DNA segments can be ligated). In some embodiments, the vector is a viral 25 vector, wherein additional DNA segments can be ligated into the viral genome. Expression vectors include, but are not limited to, plasmids, cosmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus and tobacco mosaic virus, yeast artificial chromosomes (YACs), Epstein-Barr (EBV)-derived episomes, and other expression vectors known in the art.

30 The present disclosure also provides compositions comprising any one or more of the inhibitory nucleic acid molecules. In some embodiments, the composition is a pharmaceutical composition. In some embodiments, the compositions comprise a carrier and/or excipient.

Examples of carriers include, but are not limited to, poly(lactic acid) (PLA) microspheres, poly(D,L-lactic-coglycolic-acid) (PLGA) microspheres, liposomes, micelles, inverse micelles, lipid cochleates, and lipid microtubules. A carrier may comprise a buffered salt solution such as PBS, HBSS, etc.

5 In some embodiments, the ANGPTL3 inhibitor comprises a nuclease agent that induces one or more nicks or double-strand breaks at a recognition sequence(s) or a DNA-binding protein that binds to a recognition sequence within an ANGPTL3 genomic nucleic acid molecule. The recognition sequence can be located within a coding region of the ANGPTL3 gene, or within regulatory regions that influence the expression of the gene. A recognition sequence of the
10 DNA-binding protein or nuclease agent can be located in an intron, an exon, a promoter, an enhancer, a regulatory region, or any non-protein coding region. The recognition sequence can include or be proximate to the start codon of the ANGPTL3 gene. For example, the recognition sequence can be located about 10, about 20, about 30, about 40, about 50, about 100, about 200, about 300, about 400, about 500, or about 1,000 nucleotides from the start codon. As
15 another example, two or more nuclease agents can be used, each targeting a nuclease recognition sequence including or proximate to the start codon. As another example, two nuclease agents can be used, one targeting a nuclease recognition sequence including or proximate to the start codon, and one targeting a nuclease recognition sequence including or proximate to the stop codon, wherein cleavage by the nuclease agents can result in deletion of
20 the coding region between the two nuclease recognition sequences. Any nuclease agent that induces a nick or double-strand break into a desired recognition sequence can be used in the methods and compositions disclosed herein. Any DNA-binding protein that binds to a desired recognition sequence can be used in the methods and compositions disclosed herein.

 Suitable nuclease agents and DNA-binding proteins for use herein include, but are not
25 limited to, zinc finger protein or zinc finger nuclease (ZFN) pair, Transcription Activator-Like Effector (TALE) protein or Transcription Activator-Like Effector Nuclease (TALEN), or Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)/CRISPR-associated (Cas) systems. The length of the recognition sequence can vary, and includes, for example, recognition sequences that are about 30-36 bp for a zinc finger protein or ZFN pair, about 15-18 bp for each
30 ZFN, about 36 bp for a TALE protein or TALEN, and about 20 bp for a CRISPR/Cas guide RNA.

 In some embodiments, CRISPR/Cas systems can be used to modify an ANGPTL3 genomic nucleic acid molecule within a cell. The methods and compositions disclosed herein

can employ CRISPR-Cas systems by utilizing CRISPR complexes (comprising a guide RNA (gRNA) complexed with a Cas protein) for site-directed cleavage of ANGPTL3 nucleic acid molecules.

Cas proteins generally comprise at least one RNA recognition or binding domain that can interact with gRNAs. Cas proteins can also comprise nuclease domains (such as, for example, DNase or RNase domains), DNA binding domains, helicase domains, protein-protein interaction domains, dimerization domains, and other domains. Suitable Cas proteins include, for example, a wild type Cas9 protein and a wild type Cpf1 protein (such as, for example, FnCpf1). A Cas protein can have full cleavage activity to create a double-strand break in an ANGPTL3 genomic nucleic acid molecule or it can be a nickase that creates a single-strand break in an ANGPTL3 genomic nucleic acid molecule. Additional examples of Cas proteins include, but are not limited to, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, and homologs or modified versions thereof. In some embodiments, a Cas system, such as Cas12a, can have multiple gRNAs encoded into a single crRNA. Cas proteins can also be operably linked to heterologous polypeptides as fusion proteins. For example, a Cas protein can be fused to a cleavage domain, an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. Cas proteins can be provided in any form. For example, a Cas protein can be provided in the form of a protein, such as a Cas protein complexed with a gRNA. Alternately, a Cas protein can be provided in the form of a nucleic acid molecule encoding the Cas protein, such as an RNA or DNA.

In some embodiments, targeted genetic modifications of ANGPTL3 genomic nucleic acid molecules can be generated by contacting a cell with a Cas protein and one or more gRNAs that hybridize to one or more gRNA recognition sequences within a target genomic locus in the ANGPTL3 genomic nucleic acid molecule. The gRNA recognition sequence can include or be proximate to the start codon of an ANGPTL3 genomic nucleic acid molecule or the stop codon of an ANGPTL3 genomic nucleic acid molecule. For example, the gRNA recognition sequence can be located from about 10, from about 20, from about 30, from about 40, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the start codon or the stop codon.

The gRNA recognition sequences within a target genomic locus in an ANGPTL3 genomic nucleic acid molecule are located near a Protospacer Adjacent Motif (PAM) sequence, which is a 2-6 base pair DNA sequence immediately following the DNA sequence targeted by the Cas9 nuclease. The canonical PAM is the sequence 5'-NGG-3' where "N" is any nucleobase followed by two guanine ("G") nucleobases. gRNAs can transport Cas9 to anywhere in the genome for gene editing, but no editing can occur at any site other than one at which Cas9 recognizes PAM. In addition, 5'-NGA-3' can be a highly efficient non-canonical PAM for human cells. Generally, the PAM is about 2-6 nucleotides downstream of the DNA sequence targeted by the gRNA. The PAM can flank the gRNA recognition sequence. In some embodiments, the gRNA recognition sequence can be flanked on the 3' end by the PAM. In some embodiments, the gRNA recognition sequence can be flanked on the 5' end by the PAM. For example, the cleavage site of Cas proteins can be about 1 to about 10, about 2 to about 5 base pairs, or three base pairs upstream or downstream of the PAM sequence. In some embodiments (such as when Cas9 from *S. pyogenes* or a closely related Cas9 is used), the PAM sequence of the non-complementary strand can be 5'-NGG-3', where N is any DNA nucleotide and is immediately 3' of the gRNA recognition sequence of the non-complementary strand of the target DNA. As such, the PAM sequence of the complementary strand would be 5'-CCN-3', where N is any DNA nucleotide and is immediately 5' of the gRNA recognition sequence of the complementary strand of the target DNA.

A gRNA is an RNA molecule that binds to a Cas protein and targets the Cas protein to a specific location within an ANGPTL3 genomic nucleic acid molecule. An exemplary gRNA is a gRNA effective to direct a Cas enzyme to bind to or cleave an ANGPTL3 genomic nucleic acid molecule, wherein the gRNA comprises a DNA-targeting segment that hybridizes to a gRNA recognition sequence within the ANGPTL3 genomic nucleic acid molecule. Exemplary gRNAs comprise a DNA-targeting segment that hybridizes to a gRNA recognition sequence present within an ANGPTL3 genomic nucleic acid molecule that includes or is proximate to the start codon or the stop codon. For example, a gRNA can be selected such that it hybridizes to a gRNA recognition sequence that is located from about 5, from about 10, from about 15, from about 20, from about 25, from about 30, from about 35, from about 40, from about 45, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the start codon or located from about 5, from about 10, from about 15, from about 20, from about 25, from about 30, from about 35, from about 40, from about

45, from about 50, from about 100, from about 200, from about 300, from about 400, from about 500, or from about 1,000 nucleotides of the stop codon. Suitable gRNAs can comprise from about 17 to about 25 nucleotides, from about 17 to about 23 nucleotides, from about 18 to about 22 nucleotides, or from about 19 to about 21 nucleotides. In some embodiments, the gRNAs can comprise 20 nucleotides.

The Cas protein and the gRNA form a complex, and the Cas protein cleaves the target ANGPTL3 genomic nucleic acid molecule. The Cas protein can cleave the nucleic acid molecule at a site within or outside of the nucleic acid sequence present in the target ANGPTL3 genomic nucleic acid molecule to which the DNA-targeting segment of a gRNA will bind. For example, formation of a CRISPR complex (comprising a gRNA hybridized to a gRNA recognition sequence and complexed with a Cas protein) can result in cleavage of one or both strands in or near (such as, for example, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the nucleic acid sequence present in the ANGPTL3 genomic nucleic acid molecule to which a DNA-targeting segment of a gRNA will bind.

Such methods can result, for example, in an ANGPTL3 genomic nucleic acid molecule in which a region of the ANGPTL3 genomic nucleic acid molecule is disrupted, the start codon is disrupted, the stop codon is disrupted, or the coding sequence is disrupted or deleted. Optionally, the cell can be further contacted with one or more additional gRNAs that hybridize to additional gRNA recognition sequences within the target genomic locus in the ANGPTL3 genomic nucleic acid molecule. By contacting the cell with one or more additional gRNAs (such as, for example, a second gRNA that hybridizes to a second gRNA recognition sequence), cleavage by the Cas protein can create two or more double-strand breaks or two or more single-strand breaks.

In some embodiments, the methods of treatment and/or prevention further comprise detecting the presence or absence of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule in a biological sample from the subject. In some embodiments, the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can be any of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein.

The present disclosure also provides methods of treating a subject with a kidney disease therapeutic agent, wherein the subject has a kidney disease or is at risk of developing a kidney disease, wherein the kidney disease is not nephrotic syndrome. In some embodiments,

the methods comprise determining whether the subject has an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule by obtaining or having obtained a biological sample from the subject, and performing or having performed a sequence analysis on the biological sample to determine if the subject has a genotype comprising the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. In some embodiments, the methods further comprise administering or continuing to administer the kidney disease therapeutic agent in a standard dosage amount to a subject that is ANGPTL3 reference, and/or administering an ANGPTL3 inhibitor to the subject. In some embodiments, the methods further comprise administering or continuing to administer the kidney disease therapeutic agent in an amount that is the same as or less than a standard dosage amount to a subject that is heterozygous for the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, and/or administering an ANGPTL3 inhibitor to the subject. In some embodiments, the methods further comprise administering or continuing to administer the kidney disease therapeutic agent in an amount that is the same as or less than a standard dosage amount to a subject that is homozygous for the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. The presence of a genotype having the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule indicates the subject has a decreased risk of developing a kidney disease. In some embodiments, the subject is ANGPTL3 reference. In some embodiments, the subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule.

For subjects that are genotyped or determined to be either ANGPTL3 reference or heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, such subjects can be administered an ANGPTL3 inhibitor, as described herein.

Detecting the presence or absence of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule in a biological sample from a subject and/or determining whether a subject has an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the nucleic acid molecule can be present within a cell obtained from the subject.

In some embodiments, when the subject is ANGPTL3 reference, the subject is administered a kidney disease therapeutic agent in a standard dosage amount. In some

embodiments, when the subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, the subject is administered a kidney disease therapeutic agent in a dosage amount that is the same as or less than a standard dosage amount.

5 In some embodiments, the treatment and/or prevention methods comprise detecting the presence or absence of an ANGPTL3 predicted loss-of-function polypeptide in a biological sample from the subject. In some embodiments, when the subject does not have an ANGPTL3 predicted loss-of-function polypeptide, the subject is administered a kidney disease therapeutic agent in a standard dosage amount. In some embodiments, when the subject has an ANGPTL3 predicted loss-of-function polypeptide, the subject is administered a kidney disease therapeutic agent in a dosage amount that is the same as or less than a standard dosage amount.

The present disclosure also provides methods of treating a subject with a kidney disease therapeutic agent, wherein the subject has a kidney disease or is at risk of developing a kidney disease, wherein the kidney disease is not nephrotic syndrome. In some embodiments, the method comprises determining whether the subject has an ANGPTL3 predicted loss-of-
15 function polypeptide by obtaining or having obtained a biological sample from the subject, and performing or having performed an assay on the biological sample to determine if the subject has an ANGPTL3 predicted loss-of-function polypeptide. When the subject does not have an ANGPTL3 predicted loss-of-function polypeptide, the kidney disease therapeutic agent is administered or continued to be administered to the subject in a standard dosage amount, and/or an ANGPTL3 inhibitor is administered to the subject. When the subject has an ANGPTL3 predicted loss-of-function polypeptide, the kidney disease therapeutic agent is administered or continued to be administered to the subject in an amount that is the same as or less than a standard dosage amount, and/or an ANGPTL3 inhibitor is administered to the subject. The presence of an ANGPTL3 predicted loss-of-function polypeptide indicates the subject has a
25 decreased risk of developing a kidney disease. In some embodiments, the subject has an ANGPTL3 predicted loss-of-function polypeptide. In some embodiments, the subject does not have an ANGPTL3 predicted loss-of-function polypeptide.

The present disclosure also provides methods of preventing a subject from developing a kidney disease by administering a kidney disease therapeutic agent, wherein the kidney
30 disease is not nephrotic syndrome. In some embodiments, the method comprises determining whether the subject has an ANGPTL3 predicted loss-of-function polypeptide by obtaining or having obtained a biological sample from the subject, and performing or having performed an

assay on the biological sample to determine if the subject has an ANGPTL3 predicted loss-of-function polypeptide. When the subject does not have an ANGPTL3 predicted loss-of-function polypeptide, the kidney disease therapeutic agent is administered or continued to be administered to the subject in a standard dosage amount, and/or an ANGPTL3 inhibitor is administered to the subject. When the subject has an ANGPTL3 predicted loss-of-function polypeptide, the kidney disease therapeutic agent is administered or continued to be administered to the subject in an amount that is the same as or less than a standard dosage amount, and/or an ANGPTL3 inhibitor is administered to the subject. The presence of an ANGPTL3 predicted loss-of-function polypeptide indicates the subject has a decreased risk of developing a kidney disease. In some embodiments, the subject has an ANGPTL3 predicted loss-of-function polypeptide. In some embodiments, the subject does not have an ANGPTL3 predicted loss-of-function polypeptide.

Detecting the presence or absence of an ANGPTL3 predicted loss-of-function polypeptide in a biological sample from a subject and/or determining whether a subject has an ANGPTL3 predicted loss-of-function polypeptide can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the polypeptide can be present within a cell obtained from the subject.

In some embodiments, the ANGPTL3 inhibitor is a small molecule. In some embodiments, the ANGPTL3 inhibitor is (12mer-)heparin (Gunn et al., J. Biol. Chem., 2021, 296, 1-12) or CAT-2003 (Liu et al., Arteriosclerosis, Thrombosis, and Vascular Biology, 2014, 34, A237). In some embodiments, the ANGPTL3 inhibitor is a vaccine. In some embodiments, the vaccine comprises a peptide corresponding to the LPL inhibitory domain of ANGPTL3. In some embodiments, the vaccine comprises a peptide having an amino acid sequence comprising amino acids 32 to 41 of ANGPTL3 (i.e., EPKSRFAMLD; SEQ ID NO:9) (see, Fukami et al., Cell Reports Med., 2021, 100446).

In some embodiments, the ANGPTL3 inhibitor is an antibody, or antigen-binding fragment thereof. In some embodiments, the antibody, or antigen-binding fragment thereof, binds specifically to human ANGPTL3. Exemplary antibodies, and fragments thereof, are disclosed in PCT Publication WO 2020/243031, which is incorporated herein by reference in its entirety.

In some embodiments, the antibody is a fully human monoclonal antibody (mAb), or antigen-binding fragment thereof, that specifically binds and neutralizes, inhibits, blocks, abrogates, reduces, or interferes with, at least one activity of ANGPTL3, in particular, human ANGPTL3 (SEQ ID NO:161). The activity of ANGPTL3 that can be neutralized, inhibited, blocked, 5 abrogated, reduced or interfered with, by the antibodies or fragments thereof of the present disclosure, includes, but is not limited to, inhibition of LPL activity, induction of angiogenesis, and the like. In some embodiments, an antibody or fragment thereof can neutralize, inhibit, block, abrogate, reduce, or interfere with, an activity of ANGPTL3 by binding to an epitope of ANGPTL3 that is directly involved in the targeted activity of ANGPTL3. In some embodiments, 10 an antibody or fragment thereof can neutralize, inhibit, block, abrogate, reduce, or interfere with, an activity of ANGPTL3 by binding to an epitope of ANGPTL3 that is not directly involved in the targeted activity of ANGPTL3, but the antibody or fragment binding thereto sterically or conformationally inhibits, blocks, abrogates, reduces, or interferes with, the targeted activity of ANGPTL3. In some embodiments, an antibody or fragment thereof binds to an epitope of 15 ANGPTL3 that is not directly involved in the targeted activity (e.g., inhibiting LPL activity, inducing angiogenesis, and the like) of ANGPTL3 (i.e., a non-blocking antibody), but the antibody or fragment binding thereto results in the enhancement of the clearance of ANGPTL3 from the circulation, compared to the clearance of ANGPTL3 in the absence of the antibody or fragment thereof, thereby indirectly inhibiting, blocking, abrogating, reducing, or interfering 20 with, an activity of ANGPTL3. Clearance of ANGPTL3 from the circulation can be particularly enhanced by combining two or more different non-blocking antibodies that do not compete with one another for specific binding to ANGPTL3.

The antibodies (Abs) can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, F(ab')₂ or scFv fragment), and 25 may be modified to affect functionality, e.g., to eliminate residual effector functions (Reddy et al., J. Immunol., 2000, 164, 1925-1933).

In some embodiments, the antibody or antigen-binding fragment of an antibody comprises a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO:2, 18, 34, 50, 66, 82, 98, 114, 130, 146 and 164, or a substantially similar sequence thereof 30 having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In some embodiments, the antibody or antigen-binding fragment thereof comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:2, 18, 34, 66, 82, 114,

and 164. In some embodiments, the antibody or an antigen-binding fragment thereof comprises a HCVR having an amino acid sequence of SEQ ID NO:66.

In some embodiments, an antibody or antigen-binding fragment of an antibody comprises a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO:10, 26, 42, 58, 74, 90, 106, 122, 138, 154 and 172, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In another embodiment, the antibody or antigen-binding portion of an antibody comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO:10, 26, 42, 74, 90, 122 and 172. In some embodiments, the antibody or antigen-binding portion of an antibody comprises a LCVR having an amino acid sequence of SEQ ID NO:74.

In some embodiments, the antibody or fragment thereof comprises a HCVR and LCVR sequence pair (HCVR/LCVR) selected from the group consisting of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154 and 164/172. In some embodiments, the antibody or fragment thereof comprises a HCVR and LCVR sequence pair selected from the group consisting of SEQ ID NO:2/10, 18/26, 34/42, 66/74, 82/90, 114/122 and 164/172. In some embodiments, the antibody or fragment thereof comprises a HCVR and LCVR sequence pair of SEQ ID NO:66/74.

In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain complementarity determining region 3 (HCDR3) amino acid sequence selected from the group consisting of SEQ ID NO:8, 24, 40, 56, 72, 88, 104, 120, 136, 152 and 170, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) amino acid sequence selected from the group consisting of SEQ ID NO:16, 32, 48, 64, 80, 96, 112, 128, 144, 160 and 178, or substantially similar sequences thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In some embodiments, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:8/16, 24/32, 40/48, 56/64, 72/80, 88/96, 104/112, 120/128, 136/144, 152/160 or 170/178. In some embodiments, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:8/16, 24/32, 40/48, 72/80, 88/96, 120/128 or 170/178. In some embodiments, the antibody or fragment thereof comprises a HCDR3/LCDR3 amino acid sequence pair comprising SEQ ID NO:72/80.

In some embodiments, the antibody or fragment thereof further comprises a heavy chain CDR1 (HCDR1) amino acid sequence selected from the group consisting of SEQ ID NO:4, 20, 36, 52, 68, 84, 100, 116, 132, 148 and 166, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a heavy chain CDR2 (HCDR2) amino acid sequence selected from the group consisting of SEQ ID NO:6, 22, 38, 54, 70, 86, 102, 118, 134, 150 and 168, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and optionally further comprises a light chain CDR1 (LCDR1) amino acid sequence selected from the group consisting of SEQ ID NO:12, 28, 44, 60, 76, 92, 108, 124, 140, 156 and 174, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and/or a light chain CDR2 (LCDR2) amino acid sequence selected from the group consisting of SEQ ID NO:14, 30, 46, 62, 78, 94, 110, 126, 142, 158 and 176, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

Amino acid sequences not depicted in the Sequence Listing are shown below.

Sequence	SEQ ID NO:
Ala Ala Ser	14
Lys Ala Ser	30
Ala Ala Ser	46
Lys Ala Ser	62
Lys Ala Ser	78
Thr Thr Ser	94
Pro Ala Ser	110
Thr Ala Ser	126
Lys Val Ser	142
Val Ala Ala	158
Lys Ala Ser	176

In some embodiments, the antibody or antigen-binding fragment thereof comprises a HCDR1/HCDR2/HCDR3 combination selected from the group consisting of SEQ ID NO:4/6/8, 20/22/24, 36/38/40, 52/54/56, 68/70/72, 84/86/88, 100/102/104, 116/118/120, 132/134/136, 148/150/152 and 166/168/170; and/or a LCDR1/LCDR2/LCDR3 combination selected from the

group consisting of SEQ ID NO:12/14/16, 28/30/32, 44/46/48, 60/62/64, 76/78/80, 92/94/96, 108/110/112, 124/126/128, 140/142/144, 156/158/160 and 174/176/178. In some embodiments, the heavy and light chain CDR amino acid sequences comprise a CDR sequence combination selected from the group consisting of SEQ ID NO:4/6/8/12/14/16,

5 20/22/24/28/30/32, 36/38/40/44/46/48, 52/54/56/60/62/64, 68/70/72/76/78/80, 84/86/88/92/94/96, 100/102/104/108/110/112, 116/118/120/124/126/128, 132/134/136/140/142/144, 148/150/152/156/158/160 and 166/168/170/174/176/178. In some embodiments, the heavy and light chain CDR amino acid sequences comprise a CDR sequence combination of SEQ ID NO: 4/6/8/12/14/16, 20/22/24/28/30/32,
10 36/38/40/44/46/48, 68/70/72/76/78/80, 84/86/88/92/94/96, 116/118/120/124/126/128 or 166/168/170/174/176/178. In some embodiments, the heavy and light chain CDR amino acid sequences comprise a CDR sequence combination of SEQ ID NO:68/70/72/76/78/80.

In some embodiments, the antibody or antigen-binding fragment thereof, which specifically binds ANGPTL3, comprises heavy and light chain CDR domains contained within
15 HCVR/LCVR pairs selected from the group consisting of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154 and 164/172. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are known in the art and can be applied to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Conventional definitions that can be applied to identify the boundaries of CDRs include
20 the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. See, e.g., Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani et al., J. Mol. Biol., 1997, 273,
25 927-948; and Martin et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 9268-9272. Public databases are also available for identifying CDR sequences within an antibody. In some embodiments, the antibody or fragment thereof comprises CDR sequences contained within a HCVR and LCVR pair of SEQ ID NO: 2/10, 18/26, 34/42, 66/74, 82/90, 114/122 or 164/172. In some embodiments, the antibody or fragment thereof comprises CDR sequences contained within a HCVR and LCVR
30 pair of SEQ ID NO:66/74.

In some embodiments, the antibody or antigen-binding fragment thereof competes for specific binding to ANGPTL3 with an antibody or antigen-binding fragment comprising heavy

and light chain CDR sequences contained in a HCVR/LCVR sequence pair of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154 or 164/172. In some embodiments, the antibody or antigen-binding fragment thereof competes for specific binding to ANGPTL3 with an antibody or fragment thereof comprising a HCVR/LCVR sequence pair of SEQ ID NO:66/74. In some embodiments, the antibody or antigen-binding fragment thereof competes for specific binding to ANGPTL3 with an antibody or fragment thereof comprising a heavy and light chain CDR sequence combination selected from the group consisting of 4/6/8/12/14/16, 20/22/24/28/30/32, 36/38/40/44/46/48, 52/54/56/60/62/64, 68/70/72/76/78/80, 84/86/88/92/94/96, 100/102/104/108/110/112, 116/118/120/124/126/128, 132/134/136/140/142/144, 148/150/152/156/158/160 and 166/168/170/174/176/178. In some embodiments, the antibody or antigen-binding fragment thereof competes for specific binding to ANGPTL3 with an antibody or fragment thereof comprising a heavy and light chain CDR sequence combination of SEQ ID NOS:68/70/72/76/78/80.

In some embodiments, the antibody or antigen-binding fragment thereof binds the same epitope on ANGPTL3 that is recognized by an antibody or fragment thereof comprising heavy and light chain CDR sequences from a HCVR/LCVR sequence pair of SEQ ID NO:2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154 or 164/172. In some embodiments, the antibody or antigen-binding fragment thereof binds the same epitope on ANGPTL3 as that recognized by the antibody or fragment thereof comprising a HCVR/LCVR sequence pair of SEQ ID NO:66/74. In some embodiments, the antibody or fragment thereof binds the same epitope on ANGPTL3 that is recognized by an antibody or fragment thereof comprising a heavy and light chain CDR sequence combination selected from the group consisting of 4/6/8/12/14/16, 20/22/24/28/30/32, 36/38/40/44/46/48, 52/54/56/60/62/64, 68/70/72/76/78/80, 84/86/88/92/94/96, 100/102/104/108/110/112, 116/118/120/124/126/128, 132/134/136/140/142/144, 148/150/152/156/158/160 and 166/168/170/174/176/178. In some embodiments, such an epitope is recognized by an antibody or fragment thereof comprising a heavy and light chain CDR sequence combination of SEQ ID NO:68/70/72/76/78/80.

In some embodiments, an isolated anti-ANGPTL3 antibody or antigen-binding fragment thereof that binds to an epitope situated within the N-terminal coiled-coil region at residues 17 to 209 of SEQ ID NO:161 and neutralizes, inhibits, abrogates, reduces or interferes

with, at least one activity of ANGPTL3. In some embodiments, the isolated antibody or antigen-binding fragment thereof specifically binds to an epitope situated within the N-terminal coiled-coil region of ANGPTL3 (SEQ ID NO:161) and neutralizes, inhibits, abrogates, reduces or interferes with, at least one activity of ANGPTL3, with the proviso that the antibody or fragment thereof does not bind to the ANGPTL3 peptide of SEQ ID NO:162 (corresponds to residues Glu32 to Leu57 of ANGPTL3 of SEQ ID NO:161). In some embodiments, the antibody or fragment thereof specifically binds to an epitope within residues 17 to 200, 17 to 100, 17 to 70, 17 to 65, 17 to 60, 17 to 57, or 17 to 50, of ANGPTL3 (SEQ ID NO:161), optionally with the proviso that the antibody or fragment thereof does not bind to the ANGPTL3 peptide of SEQ ID NO:162. In some embodiments, the antibody or fragment thereof specifically binds to an epitope within residues 40 to 200, 40 to 100, 40 to 70, 50 to 200, 50 to 100, 50 to 70, 58 to 200, 58 to 100, 58 to 70, 58 to 68, or 61 to 66, of ANGPTL3 (SEQ ID NO:161), optionally with the proviso that the antibody or fragment thereof does not bind to the ANGPTL3 peptide of SEQ ID NO:162. In some embodiments, the antibody or antibody fragment binds an epitope which may involve more than one of the enumerated epitopes or residues within the N-terminal coiled-coil region of ANGPTL3, optionally with the proviso that the antibody or fragment thereof does not bind to the ANGPTL3 peptide of SEQ ID NO:162.

The present disclosure also provides nucleic acid molecules that encode anti-ANGPTL3 antibodies or fragments thereof, in particular, any one of those described above. Recombinant expression vectors carrying these nucleic acids, and host cells, e.g., bacterial cells, such as *E. coli*, or mammalian cells, such as CHO cells, into which such vectors have been introduced, are also encompassed herein, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

In some embodiments, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145 and 163, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In some embodiments, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence of SEQ ID NO:1, 17, 33, 65, 81, 113 or 163. In some embodiments, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence of SEQ ID NO:65.

In some embodiments, an antibody or antigen-binding fragment thereof comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 25, 41, 57, 73, 89, 105, 121, 137, 153 and 171, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In some embodiments, the antibody or fragment thereof comprises a LCVR encoded by a nucleic acid sequence of SEQ ID NO:9, 25, 41, 73, 89, 121 or 171. In some embodiments, the antibody or fragment thereof comprises a LCVR encoded by a nucleic acid sequence of SEQ ID NO:73.

In some embodiments, the antibody or fragment thereof comprises a HCVR and LCVR (HCVR/LCVR) sequence pair encoded by a nucleic acid sequence pair selected from the group consisting of SEQ ID NO:1/9, 17/25, 33/41, 49/57, 65/73, 81/89, 97/105, 113/121, 129/137, 145/153 and 163/171. In some embodiments, the antibody or fragment thereof comprises a HCVR/LCVR sequence pair encoded by a nucleic acid sequence pair of SEQ ID NO:1/9, 17/25, 33/41, 65/73, 81/89, 113/121 or 163/171. In some embodiments, the antibody or fragment thereof comprises a HCVR/LCVR sequence pair encoded by a nucleic acid sequence pair of SEQ ID NO:65/73.

In some embodiments, the antibody or antigen-binding fragment thereof comprises a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:7, 23, 39, 55, 71, 87, 103, 119, 135, 151 and 169, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:15, 31, 47, 63, 79, 95, 111, 127, 143, 159 and 177, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof. In some embodiments, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair selected from the group consisting of SEQ ID NO:7/15, 23/31, 39/47, 55/63, 71/79, 87/95, 103/111, 119/127, 135/143, 151/159 and 169/177. In some embodiments, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair of SEQ ID NO:7/15, 23/31, 39/47, 71/79, 87/95, 119/127 or 169/177. In some embodiments, the antibody or fragment thereof comprises a HCDR3 and LCDR3 sequence pair encoded by the nucleic acid sequence pair of SEQ ID NO:71/79.

In some embodiments, the antibody or fragment thereof further comprises a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3,

19, 35, 51, 67, 83, 99, 115, 131, 147 and 165, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:5, 21, 37, 53, 69, 85, 101, 117, 133, 149 and 167, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and optionally further comprises a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:11, 27, 43, 59, 75, 91, 107, 123, 139, 155 and 173, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof; and/or a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:13, 29, 45, 61, 77, 93, 109, 125, 141, 157 and 175, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

Nucleotide sequences not depicted in the Sequence Listing are shown below.

Sequence	SEQ ID NO:
gctgcatcc	13
aaggcgtct	29
gctgcatcc	45
aaggcgtct	61
aaggcgtct	77
actacttcc	93
cctgcatcc	109
actgcatcc	125
aaggtttct	141
gttgcagcc	157
aaggcgtct	175

In some embodiments, the antibody or antigen-binding fragment thereof comprises a HCDR1/HCDR2/HCDR3 combination encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO:3/5/7, 19/21/23, 35/37/39, 51/53/55, 67/69/71, 83/85/87, 99/101/103, 115/117/119, 131/133/135, 147/149/151 and 165/167/169; and/or a LCDR1/LCDR2/LCDR3 combination encoded by a nucleotide sequence combination selected from the group consisting of SEQ ID NO:11/13/15, 27/29/31, 43/45/47, 59/61/63, 75/77/79,

91/93/95, 107/109/111, 123/125/127, 139/141/143, 155/157/159 and 173/175/177. In some embodiments, the antibody or fragment thereof comprises heavy and light chain CDR sequences encoded by a nucleotide sequence combination of SEQ ID NO:67/69/71/75/77/79.

In some embodiments, the anti-ANGPTL3 antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) encoded by nucleotide sequence segments derived from V_H , D_H and J_H germline sequences, and a light chain variable region (LCVR) encoded by nucleotide sequence segments derived from V_K and J_K germline sequences, wherein the HCVR and the LCVR are encoded by nucleotide sequence segments derived from a germline gene combination selected from the group consisting of: (i) V_{H3-43} , D_{H3-3} , 43, V_{K1-5} and J_{K2} ; (ii) V_{H3-11} , D_{H1-1} , J_{H4} , V_{K1-39} and J_{K4} ; (iii) V_{H3-30} , D_{H1-7} , J_{H6} , V_{K1-5} and J_{K1} ; (iv) V_{H3-30} , D_{H1-26} , J_{H6} , V_{K1-12} and J_{K3} ; (v) V_{H3-30} , D_{H3-10} , J_{H6} , V_{K1-12} and J_{K3} ; and (vi) V_{H3-23} , D_{H3-10} , J_{H4} , V_{K1-5} and J_{K1} .

In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to ANGPTL3 with an equilibrium dissociation constant (K_D) of about 7 nM or less, about 6 nM or less, about 5 nM or less, about 4 nM or less, about 3 nM or less, about 2 nM or less, or about 1 nM or less, as measured by surface plasmon resonance assay (for example, BIACORE™). In some embodiments, the antibody exhibits a K_D of about 800 pM or less, about 700 pM or less; about 600 pM or less; about 500 pM or less; about 400 pM or less; about 300 pM or less; about 200 pM or less; about 100 pM or less; or about 50 pM or less.

In some embodiments, the anti-ANGPTL3 antibodies have a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or e.g., removal of a fucose moiety to increase antibody dependent cellular cytotoxicity (ADCC) function (see, Shield et al., J. Biol. Chem., 2002, 277, 26733). In other applications, removal of N-glycosylation site may reduce undesirable immune reactions against the therapeutic antibodies, or increase affinities of the antibodies. In yet other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

The present disclosure also provides compositions comprising a combination of an antibody or antigen-binding fragment thereof and a second therapeutic agent. The second therapeutic agent may be one or more of any agent such as (1) 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, such as cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and the like; (2) inhibitors of

cholesterol uptake and/or bile acid re-absorption; (3) niacin, which increases lipoprotein catabolism; (4) fibrates or amphipathic carboxylic acids, which reduce low-density lipoprotein (LDL) level, improve high-density lipoprotein (HDL) and TG levels, and reduce the number of non-fatal heart attacks; and (5) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol, or fixed combinations such as ezetimibe plus simvastatin; a statin with a bile resin (e.g., cholestyramine, colestipol, colesevelam), a fixed combination of niacin plus a statin (e.g., niacin with lovastatin); or with other lipid lowering agents such as omega-3-fatty acid ethyl esters (for example, omacor). Furthermore, the second therapeutic agent can be one or more other inhibitors of ANGPTL3 as well as inhibitors of other molecules, such as ANGPTL4, ANGPTL5, ANGPTL6 and proprotein convertase subtilisin/kexin type 9 (PCSK9), which are involved in lipid metabolism, in particular, cholesterol and/or triglyceride homeostasis. Inhibitors of these molecules include small molecules and antibodies that specifically bind to these molecules and block their activity.

In some embodiments, the second therapeutic agent may be one or more anti-cancer agents, such as chemotherapeutic agents, anti-angiogenic agents, growth inhibitory agents, cytotoxic agents, apoptotic agents, and other agents well known in the art to treat cancer or other proliferative diseases or disorders, as well as other therapeutic agents, such as analgesics, anti-inflammatory agents, including non-steroidal anti-inflammatory drugs (NSAIDS), such as Cox-2 inhibitors, and the like, so as to ameliorate and/or reduce the symptoms accompanying the underlying cancer/tumor.

The present disclosure also provides methods of concomitantly treating a cardiovascular disease (e.g., such as atherosclerosis, aneurysm, hypertension, angina, stroke, cerebrovascular diseases, congestive heart failure, coronary artery diseases, myocardial infarction, peripheral vascular diseases, and the like) and any of the kidney diseases disclosed herein.

Examples of kidney disease therapeutic agents that treat or inhibit chronic kidney disease include, but are not limited to, erythropoietin, a diuretic (such as, for example, furosemide, bumetanide, ethacrynic acid, metolazone, and hydrochlorothiazide), a blood pressure medication, a phosphate binder, sodium bicarbonate, a cholesterol medication, and a gliflozin, or any combination thereof. In some embodiments, the therapeutic agent that treats or inhibits chronic kidney disease comprises an SGLT2 inhibitor such as, for example,

canagliflozin, dapagliflozin, empagliflozin, ipragliflozin, luseogliflozin, or tofogliflozin, or any combination thereof.

5 Examples of kidney disease therapeutic agents that treat or inhibit a kidney stone include, but are not limited to, potassium citrate, a diuretic (such as, for example, furosemide, bumetanide, ethacrynic acid, metolazone, and hydrochlorothiazide), allopurinol, acetohydroxamic acid, tamsulosin, nifedipine, d-penicillamine, tiopronin, and mercaptopropionyl glycine, or any combination thereof.

10 Examples of kidney disease therapeutic agents that treat or inhibit chronic glomerulonephritis include, but are not limited to, an angiotensin-converting enzyme (ACE) inhibitor (such as, for example, lisinopril, enalapril, captopril, benazepril, fosinopril, and quinapril), a diuretic (such as, for example, furosemide, bumetanide, ethacrynic acid, metolazone, and hydrochlorothiazide), a calcium channel blocker (such as, for example, amlodipine, nifedipine, felodipine, isradipine, verapamil, and diltiazem), a beta-adrenergic blocker (such as, for example, metoprolol, bisoprolol, esmolol, atenolol, propranolol, sotalol, 15 labetalol, pindolol, and penbutolol), an alpha-adrenergic agonist (such as, for example, clonidine, tizanidine, and dexmedetomidine), a corticosteroid (such as, for example, prednisone), and an immunosuppressant (such as, for example, cyclophosphamide), or any combination thereof.

20 Examples of kidney disease therapeutic agents that treat or inhibit nephronophthisis include, but are not limited to, erythropoietin and a blood pressure medication, or any combination thereof.

25 Examples of kidney disease therapeutic agents that treat or inhibit chronic interstitial nephritis include, but are not limited to, a corticosteroid, erythropoietin, a blood pressure medication, a statin, and a chelating agent (such as, for example, succimer and edetate calcium disodium), or a combination thereof.

30 Examples of kidney disease therapeutic agents that treat or inhibit nephrosclerosis include, but are not limited to, a diuretic (such as, for example, furosemide, bumetanide, ethacrynic acid, metolazone, and hydrochlorothiazide), an ACE inhibitor (such as, for example, lisinopril, enalapril, captopril, benazepril, fosinopril, and quinapril), an ARB (such as, for example, losartan, and valsartan), a calcium channel blocker (such as, for example, amlodipine, nifedipine, felodipine, isradipine, verapamil, and diltiazem), a beta-adrenergic blocker (such as, for example, metoprolol, bisoprolol, esmolol, atenolol, propranolol, sotalol, labetalol, pindolol,

and penbutolol), an alpha-adrenergic agonist (such as, for example, clonidine, tizanidine, and dexmedetomidine), a renin inhibitor (such as, for example, aliskiren), a vasodilator (such as, for example, minoxidil and hydralazine), and an alpha-1 blocker (such as, for example, doxazosin), or any combination thereof.

5 In some embodiments, the dose of the kidney disease therapeutic agent can be decreased by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, by about 60%, by about 70%, by about 80%, or by about 90% for subjects that are heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (i.e., a less than the standard dosage amount) compared to subjects that are ANGPTL3 reference (who may
10 receive a standard dosage amount). In some embodiments, the dose of the kidney disease therapeutic agent can be decreased by about 10%, by about 20%, by about 30%, by about 40%, or by about 50%. In addition, the subjects that are heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can be administered less frequently compared to subjects that are ANGPTL3 reference.

15 In some embodiments, the dose of the kidney disease therapeutic agent can be decreased by about 10%, by about 20%, by about 30%, by about 40%, by about 50%, for subjects that are homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule compared to subjects that are heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. In some embodiments, the dose of
20 the kidney disease therapeutic agent can be decreased by about 10%, by about 20%, by about 30%, by about 40%, or by about 50%. In addition, the dose of kidney disease therapeutic agent in subjects that are homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can be administered less frequently compared to subjects that are heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid
25 molecule.

Administration of the kidney disease therapeutic agent and/or ANGPTL3 inhibitors can be repeated, for example, after one day, two days, three days, five days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, eight weeks, two months, or three months. The repeated administration can be at the same dose or at a different dose. The
30 administration can be repeated once, twice, three times, four times, five times, six times, seven times, eight times, nine times, ten times, or more. For example, according to certain dosage

regimens a subject can receive therapy for a prolonged period of time such as, for example, 6 months, 1 year, or more.

Administration of the kidney disease therapeutic agent and/or ANGPTL3 inhibitors can occur by any suitable route including, but not limited to, parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal, or intramuscular. Pharmaceutical compositions for administration are desirably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically and pharmaceutically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. The term "pharmaceutically acceptable" means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

The terms "treat", "treating", and "treatment" and "prevent", "preventing", and "prevention" as used herein, refer to eliciting the desired biological response, such as a therapeutic and prophylactic effect, respectively. In some embodiments, a therapeutic effect comprises one or more of a decrease/reduction in a kidney disease, a decrease/reduction in the severity of a kidney disease (such as, for example, a reduction or inhibition of development of a kidney disease), a decrease/reduction in symptoms and kidney disease-related effects, delaying the onset of symptoms and kidney disease-related effects, reducing the severity of symptoms of kidney disease-related effects, reducing the number of symptoms and kidney disease-related effects, reducing the latency of symptoms and kidney disease-related effects, an amelioration of symptoms and kidney disease-related effects, reducing secondary symptoms, reducing secondary infections, preventing relapse to a kidney disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, increasing time to sustained progression, speeding recovery, or increasing efficacy of or decreasing resistance to alternative therapeutics, and/or an increased survival time of the affected host animal, following administration of the agent or composition comprising the agent. A prophylactic effect may comprise a complete or partial avoidance/inhibition or a delay of a kidney disease development/progression (such as, for example, a complete or partial avoidance/inhibition or a delay), and an increased survival time of the affected host animal, following administration of a therapeutic protocol. Treatment of a kidney disease encompasses

the treatment of a subject already diagnosed as having any form of a kidney disease at any clinical stage or manifestation, the delay of the onset or evolution or aggravation or deterioration of the symptoms or signs of a kidney disease, and/or preventing and/or reducing the severity of a kidney disease.

5 The present disclosure also provides methods of identifying a subject having an increased risk of developing a kidney disease, wherein the kidney disease is not nephrotic syndrome. In some embodiments, the method comprises determining or having determined in a biological sample obtained from the subject the presence or absence of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (such as a genomic nucleic acid molecule, mRNA molecule, and/or cDNA molecule). When the subject lacks an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (i.e., the subject is genotypically categorized as ANGPTL3 reference), then the subject has an increased risk of developing a kidney disease. When the subject has an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (i.e., the subject is heterozygous or homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule), then the subject has a decreased risk of developing a kidney disease.

 Having a single copy of ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule is more protective of a subject from developing a kidney disease than having no copies of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule.

20 Without intending to be limited to any particular theory or mechanism of action, it is believed that a single copy of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (i.e., heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule) is protective of a subject from developing a kidney disease, and it is also believed that having two copies of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (i.e., homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule) may be more protective of a subject from developing a kidney disease, relative to a subject with a single copy. Thus, in some embodiments, a single copy of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule may not be completely protective, but instead, may be partially or incompletely protective of a subject from developing a kidney disease. While not desiring to be bound by any particular theory, there may be additional factors or molecules involved in the development of a kidney disease that are still present in a subject having a single copy of an ANGPTL3 predicted loss-of-function

or missense variant nucleic acid molecule, thus resulting in less than complete protection from the development of a kidney disease.

Determining whether a subject has an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule in a biological sample from a subject and/or determining whether a subject has an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule can be carried out by any of the methods described herein. In some embodiments, these methods can be carried out *in vitro*. In some embodiments, these methods can be carried out *in situ*. In some embodiments, these methods can be carried out *in vivo*. In any of these embodiments, the nucleic acid molecule can be present within a cell obtained from the subject.

In some embodiments, when a subject is identified as having an increased risk of developing a kidney disease, the subject is administered a kidney disease therapeutic agent, and/or an ANGPTL3 inhibitor, as described herein. For example, when the subject is ANGPTL3 reference, and therefore has an increased risk of developing a kidney disease, the subject is administered an ANGPTL3 inhibitor. In some embodiments, such a subject is also administered a kidney disease therapeutic agent. In some embodiments, when the subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, the subject is administered the kidney disease therapeutic agent in a dosage amount that is the same as or less than a standard dosage amount, and is also administered an ANGPTL3 inhibitor. In some embodiments, such a subject is also administered a kidney disease therapeutic agent.

In some embodiments, when the subject is homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, the subject is administered the kidney disease therapeutic agent in a dosage amount that is the same as or less than a standard dosage amount. In some embodiments, the subject is ANGPTL3 reference. In some embodiments, the subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. In some embodiments, the subject is homozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule.

In some embodiments, any of the methods described herein can further comprise determining the subject's aggregate burden of having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, and/or an ANGPTL3 predicted loss-of-function variant polypeptide associated with a decreased risk of developing a kidney disease. The aggregate burden is the sum of all variants in the ANGPTL3 gene, which can be carried out in an association analysis with a kidney disease. In some embodiments, the subject is homozygous

for one or more ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules associated with a decreased risk of developing a kidney disease. In some embodiments, the subject is heterozygous for one or more ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules associated with a decreased risk of developing a kidney disease.

5 The result of the association analysis suggests that ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules are associated with decreased risk of developing a kidney disease. When the subject has a lower aggregate burden, the subject is at a higher risk of developing a kidney disease and the subject is administered or continued to be administered the therapeutic agent that treats, prevents, or inhibits a kidney disease in a standard dosage
10 amount, and/or an ANGPTL3 inhibitor. When the subject has a greater aggregate burden, the subject is at a lower risk of developing a kidney disease and the subject is administered or continued to be administered the kidney disease therapeutic agent in an amount that is the same as or less than the standard dosage amount. The greater the aggregate burden, the lower the risk of developing a kidney disease. The gene burden analysis can comprise any of the
15 ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein, such as those listed in Table 4.

In some embodiments, the subject's aggregate burden of having any one or more ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules represents a weighted sum of a plurality of any of the ANGPTL3 predicted loss-of-function or missense
20 variant nucleic acid molecules. In some embodiments, the aggregate burden is calculated using at least about 2, at least about 3, at least about 4, at least about 5, at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, at least about 100, at least about 120, at least about 150, at least about 200, at least about 250, at least about 300, at least about 400, at least about 500, at least about
25 1,000, at least about 10,000, at least about 100,000, or at least about or more than 1,000,000 genetic variants present in or around (up to 10 Mb) the ANGPTL3 gene where the genetic burden is the number of alleles multiplied by the association estimate with a kidney disease or related outcome for each allele (e.g., a weighted polygenic burden score). In some embodiments, when the subject has an aggregate burden above a desired threshold score, the
30 subject has a decreased risk of developing a kidney disease. In some embodiments, when the subject has an aggregate burden below a desired threshold score, the subject has an increased risk of developing a kidney disease.

In some embodiments, the aggregate burden may be divided into quintiles, e.g., top quintile, intermediate quintile, and bottom quintile, wherein the top quintile of aggregate burden corresponds to the lowest risk group and the bottom quintile of aggregate burden corresponds to the highest risk group. In some embodiments, a subject having a greater
5 aggregate burden comprises the highest weighted aggregate burdens, including, but not limited to the top 10%, top 20%, top 30%, top 40%, or top 50% of aggregate burdens from a subject population. In some embodiments, the genetic variants comprise the genetic variants having association with a kidney disease in the top 10%, top 20%, top 30%, top 40%, or top 50% of p-value range for the association. In some embodiments, each of the identified genetic variants
10 comprise the genetic variants having association with a kidney disease with p-value of no more than about 10^{-2} , about 10^{-3} , about 10^{-4} , about 10^{-5} , about 10^{-6} , about 10^{-7} , about 10^{-8} , about 10^{-9} , about 10^{-10} , about 10^{-11} , about 10^{-12} , about 10^{-13} , about 10^{-14} , about or 10^{-15} . In some embodiments, the identified genetic variants comprise the genetic variants having association with a kidney disease with p-value of less than 5×10^{-8} . In some embodiments, the identified
15 genetic variants comprise genetic variants having association with a kidney disease in high-risk subjects as compared to the rest of the reference population with odds ratio (OR) about 1.5 or greater, about 1.75 or greater, about 2.0 or greater, or about 2.25 or greater for the top 20% of the distribution; or about 1.5 or greater, about 1.75 or greater, about 2.0 or greater, about 2.25 or greater, about 2.5 or greater, or about 2.75 or greater. In some embodiments, the odds ratio
20 (OR) may range from about 1.0 to about 1.5, from about 1.5 to about 2.0, from about 2.0 to about 2.5, from about 2.5 to about 3.0, from about 3.0 to about 3.5, from about 3.5 to about 4.0, from about 4.0 to about 4.5, from about 4.5 to about 5.0, from about 5.0 to about 5.5, from about 5.5 to about 6.0, from about 6.0 to about 6.5, from about 6.5 to about 7.0, or greater than 7.0. In some embodiments, high-risk subjects comprise subjects having aggregate
25 burdens in the bottom decile, quintile, or tertile in a reference population. The threshold of the aggregate burden is determined on the basis of the nature of the intended practical application and the risk difference that would be considered meaningful for that practical application.

In some embodiments, when a subject is identified as having an increased risk of developing a kidney disease, the subject is further administered a kidney disease therapeutic
30 agent, and/or an ANGPTL3 inhibitor, as described herein. For example, when the subject is ANGPTL3 reference, and therefore has an increased risk of developing a kidney disease, the subject is administered an ANGPTL3 inhibitor. In some embodiments, such a subject is also

administered a kidney disease therapeutic agent. In some embodiments, when the subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, the subject is administered the kidney disease therapeutic agent in a dosage amount that is the same as or less than a standard dosage amount, and is also administered an

5 ANGPTL3 inhibitor. In some embodiments, the subject is ANGPTL3 reference. In some embodiments, the subject is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule. Furthermore, when the subject has a lower aggregate burden for having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, and therefore has an increased risk of developing a kidney disease, the subject is

10 administered a kidney disease therapeutic agent. In some embodiments, when the subject has a lower aggregate burden for having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, the subject is administered the kidney disease therapeutic agent in a dosage amount that is the same as or greater than the standard dosage amount administered to a subject who has a greater aggregate burden for having an ANGPTL3 predicted loss-of-

15 function or missense variant nucleic acid molecule.

The present disclosure also provides methods of detecting the presence or absence of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (i.e., a genomic nucleic acid molecule, an mRNA molecule, or a cDNA molecule produced from an mRNA molecule) in a biological sample from a subject. It is understood that gene sequences

20 within a population and mRNA molecules encoded by such genes can vary due to polymorphisms such as single-nucleotide polymorphisms.

The biological sample can be derived from any cell, tissue, or biological fluid from the subject. The biological sample may comprise any clinically relevant tissue, such as a bone marrow sample, a tumor biopsy, a fine needle aspirate, or a sample of bodily fluid, such as

25 blood, gingival crevicular fluid, plasma, serum, lymph, ascitic fluid, cystic fluid, or urine. In some cases, the sample comprises a buccal swab. The biological sample used in the methods disclosed herein can vary based on the assay format, nature of the detection method, and the tissues, cells, or extracts that are used as the sample. A biological sample can be processed differently depending on the assay being employed. For example, when detecting any an

30 ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, preliminary processing designed to isolate or enrich the biological sample for the genomic DNA can be employed. A variety of techniques may be used for this purpose. When detecting the level of

any an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, different techniques can be used enrich the biological sample with mRNA molecules. Various methods to detect the presence or level of an mRNA molecule or the presence of a particular variant genomic DNA locus can be used.

5 In some embodiments, detecting an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule in a subject comprises performing a sequence analysis on a biological sample obtained from the subject to determine whether ANGPTL3 genomic nucleic acid molecule in the biological sample, and/or an ANGPTL3 mRNA molecule in the biological sample, and/or an ANGPTL3 cDNA molecule produced from an mRNA molecule in the biological
10 sample, comprises one or more variations that cause a loss-of-function (partial or complete) or are predicted to cause a loss-of-function (partial or complete).

 In some embodiments, the methods of detecting the presence or absence of an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule (such as, for example, a genomic nucleic acid molecule, an mRNA molecule, and/or a cDNA molecule
15 produced from an mRNA molecule) in a subject, comprise performing an assay on a biological sample obtained from the subject. The assay determines whether a nucleic acid molecule in the biological sample comprises a particular nucleotide sequence.

 In some embodiments, the biological sample comprises a cell or cell lysate. Such methods can further comprise, for example, obtaining a biological sample from the subject
20 comprising an ANGPTL3 genomic nucleic acid molecule or mRNA molecule, and if mRNA, optionally reverse transcribing the mRNA into cDNA. Such assays can comprise, for example determining the identity of these positions of the particular ANGPTL3 nucleic acid molecule. In some embodiments, the method is an *in vitro* method.

 In some embodiments, the determining step, detecting step, or sequence analysis
25 comprises sequencing at least a portion of the nucleotide sequence of the ANGPTL3 genomic nucleic acid molecule, the ANGPTL3 mRNA molecule, or the ANGPTL3 cDNA molecule in the biological sample, wherein the sequenced portion comprises one or more variations that cause a loss-of-function (partial or complete) or are predicted to cause a loss-of-function (partial or complete).

30 In some embodiments, the assay comprises sequencing the entire nucleic acid molecule. In some embodiments, only an ANGPTL3 genomic nucleic acid molecule is analyzed.

In some embodiments, only an ANGPTL3 mRNA is analyzed. In some embodiments, only an ANGPTL3 cDNA obtained from ANGPTL3 mRNA is analyzed.

Alteration-specific polymerase chain reaction techniques can be used to detect mutations such as SNPs in a nucleic acid sequence. Alteration-specific primers can be used
5 because the DNA polymerase will not extend when a mismatch with the template is present.

In some embodiments, the nucleic acid molecule in the sample is mRNA and the mRNA is reverse-transcribed into a cDNA prior to the amplifying step. In some embodiments, the nucleic acid molecule is present within a cell obtained from the subject.

In some embodiments, the assay comprises contacting the biological sample with a
10 primer or probe, such as an alteration-specific primer or alteration-specific probe, that specifically hybridizes to an ANGPTL3 variant genomic sequence, variant mRNA sequence, or variant cDNA sequence and not the corresponding ANGPTL3 reference sequence under stringent conditions, and determining whether hybridization has occurred.

In some embodiments, the determining step, detecting step, or sequence analysis
15 comprises: a) amplifying at least a portion of the ANGPTL3 nucleic acid molecule that encodes the ANGPTL3 polypeptide; b) labeling the amplified nucleic acid molecule with a detectable label; c) contacting the labeled nucleic acid molecule with a support comprising an alteration-specific probe; and d) detecting the detectable label.

In some embodiments, the assay comprises RNA sequencing (RNA-Seq). In some
20 embodiments, the assays also comprise reverse transcribing mRNA into cDNA, such as by the reverse transcriptase polymerase chain reaction (RT-PCR).

In some embodiments, the methods utilize probes and primers of sufficient nucleotide length to bind to the target nucleotide sequence and specifically detect and/or identify a polynucleotide comprising an ANGPTL3 variant genomic nucleic acid molecule, variant mRNA
25 molecule, or variant cDNA molecule. The hybridization conditions or reaction conditions can be determined by the operator to achieve this result. The nucleotide length may be any length that is sufficient for use in a detection method of choice, including any assay described or exemplified herein. Such probes and primers can hybridize specifically to a target nucleotide sequence under high stringency hybridization conditions. Probes and primers may have
30 complete nucleotide sequence identity of contiguous nucleotides within the target nucleotide sequence, although probes differing from the target nucleotide sequence and that retain the ability to specifically detect and/or identify a target nucleotide sequence may be designed by

conventional methods. Probes and primers can have about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% sequence identity or complementarity with the nucleotide sequence of the target nucleic acid molecule.

5 Illustrative examples of nucleic acid sequencing techniques include, but are not limited to, chain terminator (Sanger) sequencing and dye terminator sequencing. Other methods involve nucleic acid hybridization methods other than sequencing, including using labeled primers or probes directed against purified DNA, amplified DNA, and fixed cell preparations (fluorescence in situ hybridization (FISH)). In some methods, a target nucleic acid molecule may
10 be amplified prior to or simultaneous with detection. Illustrative examples of nucleic acid amplification techniques include, but are not limited to, polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA). Other methods include, but are not limited to, ligase chain reaction, strand displacement amplification, and thermophilic SDA (tSDA).

15 In hybridization techniques, stringent conditions can be employed such that a probe or primer will specifically hybridize to its target. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target sequence to a detectably greater degree than to other non-target sequences, such as, at least 2-fold, at least 3-fold, at least 4-fold, or more over background, including over 10-fold over background. In some embodiments,
20 a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 2-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 3-fold. In some embodiments, a polynucleotide
25 primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by at least 4-fold. In some embodiments, a polynucleotide primer or probe under stringent conditions will hybridize to its target nucleotide sequence to a detectably greater degree than to other nucleotide sequences by over 10-fold over background. Stringent conditions are sequence-dependent and will be
30 different in different circumstances.

Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C., followed by a wash of 2X SSC at 50°C, are

known or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y.

(1989), 6.3.1-6.3.6. Typically, stringent conditions for hybridization and detection will be those in which the salt concentration is less than about 1.5 M Na⁺ ion, typically about 0.01 to 1.0 M Na⁺ ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (such as, for example, 10 to 50 nucleotides) and at least about 60°C for longer probes (such as, for example, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

In some embodiments, such isolated nucleic acid molecules comprise or consist of at least about 5, at least about 8, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, at least about 60, at least about 65, at least about 70, at least about 75, at least about 80, at least about 85, at least about 90, at least about 95, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000, at least about 2000, at least about 3000, at least about 4000, or at least about 5000

nucleotides. In some embodiments, such isolated nucleic acid molecules comprise or consist of at least about 5, at least about 8, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18, at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, or at least about 25 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consist of at least about 18 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consists of at least about 15 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 10 to about 35, from about 10 to about 30, from about 10 to about 25, from about 12 to about 30, from about 12 to about 28, from about 12 to about 24, from about 15 to about 30, from about 15 to about 25, from about 18 to about 30, from about 18 to about 25, from about 18 to about 24, or from about 18 to about 22 nucleotides. In some embodiments, the isolated nucleic acid

molecules consist of or comprise from about 18 to about 30 nucleotides. In some embodiments, the isolated nucleic acid molecules comprise or consist of at least about 15 nucleotides to at least about 35 nucleotides.

In some embodiments, such isolated nucleic acid molecules hybridize to ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules (such as genomic nucleic acid molecules, mRNA molecules, and/or cDNA molecules) under stringent conditions. Such nucleic acid molecules can be used, for example, as probes, primers, alteration-specific probes, or alteration-specific primers as described or exemplified herein, and include, without limitation primers, probes, antisense RNAs, shRNAs, and siRNAs, each of which is described in more detail elsewhere herein, and can be used in any of the methods described herein.

In some embodiments, the isolated nucleic acid molecules hybridize to at least about 15 contiguous nucleotides of a nucleic acid molecule that is at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% identical to ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 100 nucleotides, or from about 15 to about 35 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 100 nucleotides. In some embodiments, the isolated nucleic acid molecules consist of or comprise from about 15 to about 35 nucleotides.

In some embodiments, the alteration-specific probes and alteration-specific primers comprise DNA. In some embodiments, the alteration-specific probes and alteration-specific primers comprise RNA.

In some embodiments, the probes and primers described herein (including alteration-specific probes and alteration-specific primers) have a nucleotide sequence that specifically hybridizes to any of the nucleic acid molecules disclosed herein, or the complement thereof. In some embodiments, the probes and primers specifically hybridize to any of the nucleic acid molecules disclosed herein under stringent conditions.

In some embodiments, the primers, including alteration-specific primers, can be used in second generation sequencing or high throughput sequencing. In some instances, the primers, including alteration-specific primers, can be modified. In particular, the primers can comprise various modifications that are used at different steps of, for example, Massive Parallel

Signature Sequencing (MPSS), Polony sequencing, and 454 Pyrosequencing. Modified primers can be used at several steps of the process, including biotinylated primers in the cloning step and fluorescently labeled primers used at the bead loading step and detection step. Polony sequencing is generally performed using a paired-end tags library wherein each molecule of

5 DNA template is about 135 bp in length. Biotinylated primers are used at the bead loading step and emulsion PCR. Fluorescently labeled degenerate nonamer oligonucleotides are used at the detection step. An adaptor can contain a 5'-biotin tag for immobilization of the DNA library onto streptavidin-coated beads.

The probes and primers described herein can be used to detect a nucleotide variation

10 within any of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein. The primers described herein can be used to amplify any ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, or a fragment thereof.

In the context of the disclosure "specifically hybridizes" means that the probe or primer (such as, for example, the alteration-specific probe or alteration-specific primer) does

15 not hybridize to a nucleic acid sequence encoding an ANGPTL3 reference genomic nucleic acid molecule, an ANGPTL3 reference mRNA molecule, and/or an ANGPTL3 reference cDNA molecule.

In some embodiments, the probes (such as, for example, an alteration-specific probe) comprise a label. In some embodiments, the label is a fluorescent label, a radiolabel, or biotin.

The present disclosure also provides supports comprising a substrate to which any one

20 or more of the probes disclosed herein is attached. Solid supports are solid-state substrates or supports with which molecules, such as any of the probes disclosed herein, can be associated. A form of solid support is an array. Another form of solid support is an array detector. An array detector is a solid support to which multiple different probes have been coupled in an array,

25 grid, or other organized pattern. A form for a solid-state substrate is a microtiter dish, such as a standard 96-well type. In some embodiments, a multiwell glass slide can be employed that normally contains one array per well.

The genomic nucleic acid molecules, mRNA molecules, and cDNA molecules can be from any organism. For example, the genomic nucleic acid molecules, mRNA molecules, and

30 cDNA molecules can be human or an ortholog from another organism, such as a non-human mammal, a rodent, a mouse, or a rat. It is understood that gene sequences within a population can vary due to polymorphisms such as single-nucleotide polymorphisms.

Also provided herein are functional polynucleotides that can interact with the disclosed nucleic acid molecules. Examples of functional polynucleotides include, but are not limited to, antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional polynucleotides can act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional polynucleotides can possess a *de novo* activity independent of any other molecules.

The isolated nucleic acid molecules disclosed herein can comprise RNA, DNA, or both RNA and DNA. The isolated nucleic acid molecules can also be linked or fused to a heterologous nucleic acid sequence, such as in a vector, or a heterologous label. For example, the isolated nucleic acid molecules disclosed herein can be within a vector or as an exogenous donor sequence comprising the isolated nucleic acid molecule and a heterologous nucleic acid sequence. The isolated nucleic acid molecules can also be linked or fused to a heterologous label. The label can be directly detectable (such as, for example, fluorophore) or indirectly detectable (such as, for example, hapten, enzyme, or fluorophore quencher). Such labels can be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Such labels include, for example, radiolabels, pigments, dyes, chromogens, spin labels, and fluorescent labels. The label can also be, for example, a chemiluminescent substance; a metal-containing substance; or an enzyme, where there occurs an enzyme-dependent secondary generation of signal. The term "label" can also refer to a "tag" or hapten that can bind selectively to a conjugated molecule such that the conjugated molecule, when added subsequently along with a substrate, is used to generate a detectable signal. For example, biotin can be used as a tag along with an avidin or streptavidin conjugate of horseradish peroxidase (HRP) to bind to the tag, and examined using a calorimetric substrate (such as, for example, tetramethylbenzidine (TMB)) or a fluorogenic substrate to detect the presence of HRP. Exemplary labels that can be used as tags to facilitate purification include, but are not limited to, myc, HA, FLAG or 3XFLAG, 6Xhis or polyhistidine, glutathione-S-transferase (GST), maltose binding protein, an epitope tag, or the Fc portion of immunoglobulin. Numerous labels include, for example, particles, fluorophores, haptens, enzymes and their calorimetric, fluorogenic and chemiluminescent substrates and other labels.

Percent identity (or percent complementarity) between particular stretches of nucleotide sequences within nucleic acid molecules or amino acid sequences within polypeptides can be determined routinely using BLAST programs (basic local alignment search

tools) and PowerBLAST programs (Altschul *et al.*, J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). Herein, if reference is made to percent sequence identity, the higher percentages of sequence identity are preferred over the lower ones.

The present disclosure also provides kidney disease therapeutic agents for use in the treatment and/or prevention of a kidney disease in a subject having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome. Any of the kidney disease therapeutic agents described herein can be used herein. Any of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein can be used herein.

The present disclosure also provides uses of kidney disease therapeutic agents for use in the preparation of a medicament for treating and/or preventing a kidney disease in a subject having an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome. Any of the kidney disease therapeutic agents described herein can be used herein. Any of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein can be used herein.

The present disclosure also provides ANGPTL3 inhibitors for use in the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome. Any of the ANGPTL3 inhibitors described herein can be used herein. Any of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein can be used herein.

The present disclosure also provides ANGPTL3 inhibitors in the preparation of a medicament for treating and/or preventing a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for an ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome. Any of the ANGPTL3 inhibitors described herein can be used herein. Any of the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecules disclosed herein can be used herein.

All patent documents, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the

same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise, if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the present disclosure can be used in combination with any other feature, step, element, embodiment, or aspect unless specifically indicated otherwise. Although the present disclosure has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

The following examples are provided to describe the embodiments in greater detail. They are intended to illustrate, not to limit, the claimed embodiments. The following examples provide those of ordinary skill in the art with a disclosure and description of how the compounds, compositions, articles, devices and/or methods described herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of any claims. Efforts have been made to ensure accuracy with respect to numbers (such as, for example, amounts, temperature, etc.), but some errors and deviations may be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Examples

Example 1: Loss-of-function of ANGPTL3 is Associated with Improved Kidney Function and Decreased Risk of Kidney Diseases

To identify genetic factors contributing to predisposition for or protection against chronic kidney disease, exome sequencing was performed in 546,327 participants from the UK Biobank cohort (UKB) and Geisinger Health System DiscovEHR study (GHS). Associations with estimated glomerular filtration rate (eGFR), a widely used biomarker of kidney function in which higher levels indicate better function, were estimated for the burden of rare loss-of-function and missense variants in *ANGPTL3* identified by exome sequencing. The rare loss-of-

function and missense variants in *ANGPTL3* were subsequently evaluated for association with clinical diagnoses of kidney disease in UKB and GHS.

In this analysis, the burden of rare (alternative allele frequency [AAF] < 0.1%) predicted loss-of-function (pLOF) or missense genetic variants in *ANGPTL3* was strongly associated with increased eGFR (see, Table 1). Importantly, the burden of pLOF or missense genetic variants was associated with increased eGFR regardless of whether eGFR was calculated using serum creatinine (eGFR_{creatinine}) or serum cystatin (eGFR_{cystatin}), indicating an effect of *ANGPTL3* rare variants on kidney function and not creatinine or cystatin metabolism *per se*.

Table 1: Associations with increased eGFR levels for the burden of rare (AAF < 0.1%) pLOF or missense variants in the *ANGPTL3* gene

Genetic exposure	Outcome	Effect per allele [95% CI]	Pval	Genotype counts, Ref Het Hom	AAF
<i>ANGPTL3</i> pLOF or any missense variants	eGFR _{creatinine}	0.04 [0.02,0.07] SD, 0.75 [0.36,1.14] mL/min	1.7x10 ⁻⁴	541,571 4,750 6	0.0044
<i>ANGPTL3</i> pLOF or 5/5 missense variants	eGFR _{creatinine}	0.05 [0.02,0.08] SD, 0.86 [0.32,1.40] mL/min	0.002	543,886 2,437 4	0.0022
<i>ANGPTL3</i> pLOF or any missense variants	eGFR _{cystatin}	0.03 [0.01,0.06] SD, 0.52 [0.11,0.92] mL/min	0.013	429,440 3,684 5	0.0043
<i>ANGPTL3</i> pLOF or 5/5 missense variants	eGFR _{cystatin}	0.05 [0.01,0.08] SD, 0.73 [0.16,1.3] mL/min	0.012	431,279 1,847 3	0.0021

Abbreviations: CI, confidence interval; SD, standard deviation; mL/min, milliliters per minute; AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of rare coding variant (defined in 'Genetic exposure') in *ANGPTL3*; Hom, homozygous carrier of rare coding variant (defined in 'Genetic exposure') in *ANGPTL3*; pLOF, predicted loss of function; missense (5/5), missense variant predicted to be deleterious by 5 out of 5 *in silico* prediction algorithms; eGFR_{creatinine}, estimated glomerular filtration rate calculated using serum creatinine; eGFR_{cystatin}, estimated glomerular filtration rate calculated using serum cystatin.

An association for rare *ANGPTL3* pLOF variants alone (AAF <1%, excluding missense variants) with increased eGFR was also observed (see, Table 2), indicating that the association for rare pLOF plus missense variants reflects a loss-of-function in *ANGPTL3*.

Table 2: Associations with increased eGFR levels for the burden of rare (AAF < 1%) pLOF variants in the *ANGPTL3* gene

Genetic exposure	Outcome	Effect per allele [95% CI]	Pval	Genotype counts, Ref Het Hom	AAF
<i>ANGPTL3</i> pLOF variants	eGFR _{creatinine}	0.06[0.02,0.11] SD, 1.03 [0.26,1.79] mL/min	0.008	545,101 1,225 1	0.00112

Abbreviations: CI, confidence interval; SD, standard deviation; mL/min, milliliters per minute;

5 AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of rare pLOF variant in *ANGPTL3*; Hom, homozygous carrier of rare pLOF variant in *ANGPTL3*; pLOF, predicted loss of function; eGFR_{creatinine}, estimated glomerular filtration rate calculated using serum creatinine.

10 **Example 2: Loss-of-Function in *ANGPTL3* is Associated with Protection from Chronic Kidney Disease**

The association of rare coding variants in *ANGPTL3* was next estimated with kidney disease outcomes. The burdens of *ANGPTL3* rare coding variants were associated with protection against reduced eGFR, microalbuminuria (increased excretion of albumin protein in the urine, an orthogonal marker of kidney damage) any kidney disease (a broadly-defined composite outcome including kidney diseases of different types and etiology), any chronic kidney disease including or excluding microalbuminuria, and severe chronic kidney disease such as stage 5+ or end-stage renal disease (see, Table 3). Heterozygous carriers of these genetic variants had 7% to 45% lower odds of kidney disease compared to non-carriers. Therefore, loss-of-function or deleterious missense variation in *ANGPTL3* is associated with protection against various types and severities of chronic kidney disease in humans.

Table 3: Association of any missense, predicted deleterious missense variants or predicted loss of function variants in *ANGPTL3* with protection from reduced eGFR, microalbuminuria, any kidney disease, chronic kidney disease, or end-stage renal disease in UK Biobank and Geisinger Health System

Genetic exposure	Outcome	OR [95% CI]	Pval	Genotype counts in cases, Ref Het Hom	Genotype counts in	AAF
------------------	---------	-------------	------	---------------------------------------	--------------------	-----

					controls, Ref Het Hom	
ANGPTL3 pLOF variants, AAF <1%	1	0.66 [0.45,0.96]	0.030	22,530 41 0	310,675 709 1	0.00113
	2	0.73 [0.56,0.96]	0.026	31,569 58 0	489,971 1,097 1	0.00111
	3	0.75 [0.56,1.00]	0.047	40,821 75 0	217,259 516 0	0.00114
ANGPTL3 pLOF or any missense variants (AAF <0.1%)	4	0.93 [0.87,0.99]	0.028	230,784 1,906 1	310,209 2,835 5	0.00435
	5	0.83 [0.71,0.97]	0.016	22,553 176 2	165,744 1,509 1	0.00445
	6	0.90 [0.82,0.99]	0.033	83,825 706 2	216,704 2,012 1	0.00449
	7	0.88 [0.78,0.98]	0.026	59,169 479 2	216,704 2,012 1	0.00449
	8	0.55 [0.35,0.85]	0.008	3,120 13 0	216,864 1,979 0	0.00449
	9	0.59 [0.37, 0.93]	0.022	2,853 12 0	216,864 1,979 0	0.0044
ANGPTL3 pLOF or 5/5 missense variants (AAF < 0.1%)	10	0.91 [0.83,1.00]	0.045	231,715 976 0	311,589 1,456 4	0.00224
	11	0.79 [0.64,0.98]	0.029	22,645 85 1	166,501 752 1	0.00221
	12	0.85 [0.74,0.98]	0.021	84,179 353 1	217,673 1,043 1	0.00231
	13	0.80 [0.66,0.97]	0.024	31,535 124 0	490,856 2,187 3	0.00221
	14	0.80 [0.65,0.98]	0.028	40,736 160 0	216,756 1,019 0	0.00228
	15	0.81 [0.69,0.95]	0.009	59,415 234 1	217,673 1,043 1	0.0023

Outcomes: 1 = CKD (eGFR diagnosis); 2 = CKD (EHR diagnosis); 3 = CKD, any; 4 = Reduced eGFR; 5 = Microalbuminuria; 6 = Any kidney disease; 7 = CKD, any including microalbuminuria; 8 = CKD, stage 5+; 9 = End stage kidney disease; 10 = Reduced eGFR; 11 = Microalbuminuria; 12 = Any kidney disease; 13 = CKD (EHR diagnosis); 14 = CKD, any; 15 = CKD, any including

5 microalbuminuria

Abbreviations: CI, confidence interval; AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of rare coding variant (defined in 'Genetic exposure') in *ANGPTL3*; Hom, homozygous carrier of rare coding variant (defined in 'Genetic exposure') in *ANGPTL3*; pLOF, predicted loss of function; missense (5/5), missense variant

10 predicted to be deleterious by 5 out of 5 *in silico* prediction algorithms; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; EHR, electronic health record; Reduced eGFR, eGFR < 90 mL/min; CKD (eGFR diagnosis), eGFR < 60 mL/min (cases) vs eGFR > 90 mL/min (controls); all other diseases defined in Table 5.

The associations between *ANGPTL3* and kidney phenotypes were driven by multiple rare pLOF or missense variants in the *ANGPTL3* gene (see, Table 4).

Table 4: Missense or pLOF variants in *ANGPTL3* that were identified by exome sequencing and included in the gene burden association analyses

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62597582:C:T	MS (0/5)	c.16C>T	p.Leu6Phe	No	No	Yes	1.2E-06
1:62597595:T:C	MS (3/5)	c.29T>C	p.Ile10Thr	No	No	Yes	2.4E-06
1:62597600:C:T	MS (1/5)	c.34C>T	p.Pro12Ser	No	No	Yes	4.4E-06
1:62597606:G:C	MS (3/5)	c.40G>C	p.Val14Leu	No	No	Yes	2.4E-04
1:62597607:T:A	MS (4/5)	c.41T>A	p.Val14Asp	No	No	Yes	1.2E-06
1:62597607:T:C	MS (2/5)	c.41T>C	p.Val14Ala	No	No	Yes	2.4E-05
1:62597612:T:C	MS (1/5)	c.46T>C	p.Ser16Pro	No	No	Yes	4.9E-06
1:62597616:C:G	MS (4/5)	c.50C>G	p.Ser17Cys	No	No	Yes	6.5E-05
1:62597619:G:A	MS (0/5)	c.53G>A	p.Arg18Lys	No	No	Yes	1.2E-06
1:62597622:T:C	MS (0/5)	c.56T>C	p.Ile19Thr	No	No	Yes	4.9E-06
1:62597627:C:A	MS (1/5)	c.61C>A	p.Gln21Lys	No	No	Yes	3.8E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62597628:A:G	MS (1/5)	c.62A>G	p.Gln21Arg	No	No	Yes	2.4E-06
1:62597629:A:C	MS (1/5)	c.63A>C	p.Gln21His	No	No	Yes	1.5E-04
1:62597632:C:G	MS (1/5)	c.66C>G	p.Asp22Glu	No	No	Yes	2.4E-04
1:62597636:T:C	MS (0/5)	c.70T>C	p.Ser24Pro	No	No	Yes	9.5E-06
1:62597637:C:T	MS (0/5)	c.71C>T	p.Ser24Leu	No	No	Yes	4.4E-06
1:62597639:T:C	MS (0/5)	c.73T>C	p.Ser25Pro	No	No	Yes	1.3E-05
1:62597642:T:C	MS (0/5)	c.76T>C	p.Phe26Leu	No	No	Yes	2.7E-04
1:62597649:C:G	MS (4/5)	c.83C>G	p.Ser28Cys	No	No	Yes	1.2E-06
1:62597654:T:C	MS (0/5)	c.88T>C	p.Ser30Pro	No	No	Yes	1.2E-06
1:62597660:G:A	MS (4/5)	c.94G>A	p.Glu32Lys	No	No	Yes	5.9E-05
1:62597660:G:C	MS (3/5)	c.94G>C	p.Glu32Gln	No	No	Yes	1.9E-06
1:62597663:C:A	MS (1/5)	c.97C>A	p.Pro33Thr	No	No	Yes	1.2E-06
1:62597664:C:T	MS (2/5)	c.98C>T	p.Pro33Leu	No	No	Yes	1.2E-06
1:62597668:A:T	MS (3/5)	c.102A>T	p.Lys34Asn	No	No	Yes	5.1E-05
1:62597669:T:C	MS (1/5)	c.103T>C	p.Ser35Pro	No	No	Yes	3.6E-06
1:62597681:A:G	MS (3/5)	c.115A>G	p.Met39Val	No	No	Yes	1.2E-06
1:62597682:T:C	MS (4/5)	c.116T>C	p.Met39Thr	No	No	Yes	4.9E-06
1:62597696:A:C	MS (2/5)	c.130A>C	p.Lys44Gln	No	No	Yes	1.2E-06
1:62597702:T:G	MS (4/5)	c.136T>G	p.Leu46Val	No	No	Yes	1.2E-06
1:62597730:A:G	MS (3/5)	c.164A>G	p.His55Arg	No	No	Yes	2.4E-04
1:62597739:A:G	MS (1/5)	c.173A>G	p.Lys58Arg	No	No	Yes	1.2E-06
1:62597751:A:C	MS (4/5)	c.185A>C	p.His62Pro	No	No	Yes	8.8E-06
1:62597772:A:G	MS (3/5)	c.206A>G	p.Asn69Ser	No	No	Yes	1.2E-06
1:62597780:T:C	MS (3/5)	c.214T>C	p.Phe72Leu	No	No	Yes	1.2E-06
1:62597782:T:G	MS (3/5)	c.216T>G	p.Phe72Leu	No	No	Yes	4.4E-06
1:62597783:C:A	MS (1/5)	c.217C>A	p.Gln73Lys	No	No	Yes	1.2E-04
1:62597792:A:T	MS (2/5)	c.226A>T	p.Asn76Tyr	No	No	Yes	5.1E-05
1:62597798:T:C	MS (4/5)	c.232T>C	p.Phe78Leu	No	No	Yes	1.9E-06
1:62597801:G:A	MS (4/5)	c.235G>A	p.Asp79Asn	No	No	Yes	7.6E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62597804:C:G	MS (1/5)	c.238C>G	p.Gln80Glu	No	No	Yes	1.2E-05
1:62597805:A:G	MS (1/5)	c.239A>G	p.Gln80Arg	No	No	Yes	1.2E-06
1:62597806:G:C	MS (2/5)	c.240G>C	p.Gln80His	No	No	Yes	1.2E-06
1:62597808:C:A	MS (4/5)	c.242C>A	p.Ser81Tyr	No	No	Yes	1.2E-06
1:62597817:A:T	MS (4/5)	c.251A>T	p.Asp84Val	No	No	Yes	1.9E-05
1:62597823:C:T	MS (4/5)	c.257C>T	p.Ser86Leu	No	No	Yes	9.5E-06
1:62597826:T:C	MS (3/5)	c.260T>C	p.Leu87Pro	No	No	Yes	3.6E-06
1:62597837:G:A	MS (2/5)	c.271G>A	p.Glu91Lys	No	No	Yes	1.9E-06
1:62597838:A:G	MS (4/5)	c.272A>G	p.Glu91Gly	No	No	Yes	4.9E-04
1:62597848:A:C	MS (4/5)	c.282A>C	p.Glu94Asp	No	No	Yes	1.2E-06
1:62597849:G:C	MS (3/5)	c.283G>C	p.Glu95Gln	No	No	Yes	1.9E-06
1:62597852:G:A	MS (3/5)	c.286G>A	p.Glu96Lys	No	No	Yes	1.2E-06
1:62597852:G:C	MS (4/5)	c.286G>C	p.Glu96Gln	No	No	Yes	1.2E-06
1:62597857:G:C	MS (3/5)	c.291G>C	p.Lys97Asn	No	No	Yes	1.2E-06
1:62597868:G:C	MS (1/5)	c.302G>C	p.Arg101Thr	No	No	Yes	1.1E-05
1:62597869:A:T	MS (1/5)	c.303A>T	p.Arg101Ser	No	No	Yes	5.9E-05
1:62597876:T:C	MS (0/5)	c.310T>C	p.Tyr104His	No	No	Yes	2.4E-04
1:62597894:A:G	MS (4/5)	c.328A>G	p.Asn110Asp	No	No	Yes	4.0E-05
1:62597904:T:A	MS (3/5)	c.338T>A	p.Val113Glu	No	No	Yes	4.4E-06
1:62597907:A:C	MS (4/5)	c.341A>C	p.Lys114Thr	No	No	Yes	1.2E-06
1:62597913:T:C	MS (2/5)	c.347T>C	p.Met116Thr	No	No	Yes	1.2E-06
1:62597918:C:T	MS (2/5)	c.352C>T	p.Leu118Phe	No	No	Yes	2.4E-05
1:62597919:T:C	MS (3/5)	c.353T>C	p.Leu118Pro	No	No	Yes	4.4E-06
1:62597927:A:C	MS (3/5)	c.361A>C	p.Asn121His	No	No	Yes	1.2E-06
1:62597927:A:G	MS (1/5)	c.361A>G	p.Asn121Asp	No	No	Yes	4.9E-06
1:62597931:C:T	MS (1/5)	c.365C>T	p.Ser122Leu	No	No	Yes	3.6E-06
1:62597943:G:A	MS (0/5)	c.377G>A	p.Ser126Asn	No	No	Yes	2.4E-06
1:62597951:G:A	MS (3/5)	c.385G>A	p.Glu129Lys	No	No	Yes	2.0E-04
1:62597952:A:G	MS (4/5)	c.386A>G	p.Glu129Gly	No	No	Yes	2.4E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62597954:G:C	MS (4/5)	c.388G>C	p.Glu130Gln	No	No	Yes	1.2E-06
1:62597958:A:C	MS (4/5)	c.392A>C	p.Lys131Thr	No	No	Yes	1.2E-06
1:62597959:A:C	MS (3/5)	c.393A>C	p.Lys131Asn	No	No	Yes	5.9E-05
1:62597963:C:A	MS (1/5)	c.397C>A	p.Leu133Ile	No	No	Yes	1.0E-05
1:62597967:T:C	MS (4/5)	c.401T>C	p.Leu134Pro	No	No	Yes	2.4E-06
1:62597978:G:C	MS (3/5)	c.412G>C	p.Val138Leu	No	No	Yes	7.6E-06
1:62597985:A:C	MS (0/5)	c.419A>C	p.Tyr140Ser	No	No	Yes	1.2E-06
1:62597993:G:A	MS (1/5)	c.427G>A	p.Glu143Lys	No	No	Yes	4.8E-06
1:62598003:C:T	MS (1/5)	c.437C>T	p.Thr146Ile	No	No	Yes	1.2E-06
1:62598005:A:G	MS (0/5)	c.439A>G	p.Asn147Asp	No	No	Yes	1.2E-06
1:62598006:A:C	MS (0/5)	c.440A>C	p.Asn147Thr	No	No	Yes	2.5E-05
1:62598012:T:C	MS (1/5)	c.446T>C	p.Ile149Thr	No	No	Yes	1.2E-06
1:62598017:A:G	MS (1/5)	c.451A>G	p.Asn151Asp	No	No	Yes	2.7E-04
1:62598033:C:A	MS (1/5)	c.467C>A	p.Pro156Gln	No	No	Yes	2.5E-04
1:62598038:C:T	MS (1/5)	c.472C>T	p.His158Tyr	No	No	Yes	1.2E-06
1:62598041:C:A	MS (1/5)	c.475C>A	p.Pro159Thr	No	No	Yes	2.4E-04
1:62598045:A:C	MS (3/5)	c.479A>C	p.Glu160Ala	No	No	Yes	5.1E-05
1:62598046:A:C	MS (2/5)	c.480A>C	p.Glu160Asp	No	No	Yes	1.2E-06
1:62598048:T:C	MS (1/5)	c.482T>C	p.Val161Ala	No	No	Yes	1.2E-05
1:62598056:C:A	MS (4/5)	c.490C>A	p.Leu164Ile	No	No	Yes	2.4E-06
1:62598056:C:T	MS (4/5)	c.490C>T	p.Leu164Phe	No	No	Yes	8.2E-05
1:62598705:G:A	MS (2/5)	c.505G>A	p.Glu169Lys	No	No	Yes	2.4E-06
1:62598707:A:T	MS (3/5)	c.507A>T	p.Glu169Asp	No	No	Yes	5.1E-05
1:62598708:A:G	MS (1/5)	c.508A>G	p.Lys170Glu	No	No	Yes	2.4E-06
1:62598719:T:A	MS (0/5)	c.519T>A	p.Asn173Lys	No	No	Yes	1.2E-06
1:62598722:C:G	MS (1/5)	c.522C>G	p.Ser174Arg	No	No	Yes	1.2E-06
1:62598728:A:T	MS (1/5)	c.528A>T	p.Lys176Asn	No	No	Yes	2.4E-04
1:62598732:C:T	MS (4/5)	c.532C>T	p.Leu178Phe	No	No	Yes	1.2E-06
1:62598735:C:T	MS (1/5)	c.535C>T	p.Leu179Phe	No	No	Yes	1.2E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62598744:G:A	MS (3/5)	c.544G>A	p.Val182Met	No	No	Yes	2.4E-05
1:62598745:T:C	MS (4/5)	c.545T>C	p.Val182Ala	No	No	Yes	2.4E-06
1:62598747:G:A	MS (1/5)	c.547G>A	p.Glu183Lys	No	No	Yes	1.2E-06
1:62598750:G:A	MS (1/5)	c.550G>A	p.Asp184Asn	No	No	Yes	2.4E-04
1:62598751:A:C	MS (1/5)	c.551A>C	p.Asp184Ala	No	No	Yes	8.8E-06
1:62598757:A:G	MS (3/5)	c.557A>G	p.Tyr186Cys	No	No	Yes	5.7E-06
1:62598769:A:T	MS (3/5)	c.569A>T	p.Asn190Ile	No	No	Yes	9.7E-06
1:62598770:C:A	MS (2/5)	c.570C>A	p.Asn190Lys	No	No	Yes	1.2E-06
1:62598777:C:T	MS (3/5)	c.577C>T	p.His193Tyr	No	No	Yes	1.2E-06
1:62598779:T:G	MS (0/5)	c.579T>G	p.His193Gln	No	No	Yes	8.8E-06
1:62598780:A:T	MS (2/5)	c.580A>T	p.Ser194Cys	No	No	Yes	1.2E-06
1:62598782:T:A	MS (1/5)	c.582T>A	p.Ser194Arg	No	No	Yes	1.2E-06
1:62598796:T:C	MS (3/5)	c.596T>C	p.Ile199Thr	No	No	Yes	1.1E-05
1:62598802:A:G	MS (1/5)	c.602A>G	p.Asn201Ser	No	No	Yes	4.4E-06
1:62601082:C:A	MS (2/5)	c.607C>A	p.Leu203Ile	No	No	Yes	2.4E-04
1:62601090:G:T	MS (1/5)	c.615G>T	p.Arg205Ser	No	No	Yes	1.2E-06
1:62601094:A:G	MS (0/5)	c.619A>G	p.Ser207Gly	No	No	Yes	1.2E-06
1:62601098:T:G	MS (1/5)	c.623T>G	p.Ile208Ser	No	No	Yes	1.2E-06
1:62601106:C:T	MS (1/5)	c.631C>T	p.Pro211Ser	No	No	Yes	5.1E-05
1:62601107:C:T	MS (0/5)	c.632C>T	p.Pro211Leu	No	No	Yes	1.2E-06
1:62601113:A:G	MS (2/5)	c.638A>G	p.Glu213Gly	No	No	Yes	1.2E-06
1:62601115:A:T	MS (1/5)	c.640A>T	p.Ile214Phe	No	No	Yes	2.0E-04
1:62601116:T:C	MS (1/5)	c.641T>C	p.Ile214Thr	No	No	Yes	1.2E-06
1:62601122:T:C	MS (1/5)	c.647T>C	p.Leu216Pro	No	No	Yes	8.5E-06
1:62601132:G:C	MS (1/5)	c.657G>C	p.Lys219Asn	No	No	Yes	1.2E-06
1:62601133:C:A	MS (1/5)	c.658C>A	p.Pro220Thr	No	No	Yes	2.4E-06
1:62601140:C:T	MS (1/5)	c.665C>T	p.Ala222Val	No	No	Yes	2.4E-04
1:62601142:C:T	MS (2/5)	c.667C>T	p.Pro223Ser	No	No	Yes	5.1E-05
1:62601143:C:A	MS (3/5)	c.668C>A	p.Pro223Gln	No	No	Yes	4.4E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62601152:C:A	MS (2/5)	c.677C>A	p.Thr226Asn	No	No	Yes	1.2E-06
1:62601154:C:G	MS (1/5)	c.679C>G	p.Pro227Ala	No	No	Yes	4.4E-06
1:62601154:C:T	MS (1/5)	c.679C>T	p.Pro227Ser	No	No	Yes	3.6E-06
1:62601166:T:A	MS (1/5)	c.691T>A	p.Leu231Met	No	No	Yes	2.2E-05
1:62601175:A:C	MS (0/5)	c.700A>C	p.Ile234Leu	No	No	Yes	4.4E-06
1:62601176:T:C	MS (0/5)	c.701T>C	p.Ile234Thr	No	No	Yes	1.2E-06
1:62601196:G:A	MS (1/5)	c.721G>A	p.Gly241Ser	No	No	Yes	9.7E-06
1:62601778:C:T	MS (1/5)	c.731C>T	p.Ala244Val	No	No	Yes	6.7E-06
1:62601803:A:T	MS (1/5)	c.756A>T	p.Arg252Ser	No	No	Yes	1.2E-06
1:62601813:A:G	MS (0/5)	c.766A>G	p.Thr256Ala	No	No	Yes	8.7E-05
1:62601823:T:C	MS (1/5)	c.776T>C	p.Met259Thr	No	No	Yes	9.1E-04
1:62601823:T:G	MS (2/5)	c.776T>G	p.Met259Arg	No	No	Yes	9.5E-06
1:62601825:T:C	MS (4/5)	c.778T>C	p.Tyr260His	No	No	Yes	3.6E-06
1:62601836:A:C	MS (2/5)	c.789A>C	p.Arg263Ser	No	No	Yes	1.8E-06
1:62601836:A:T	MS (2/5)	c.789A>T	p.Arg263Ser	No	No	Yes	1.8E-05
1:62601837:C:T	MS (4/5)	c.790C>T	p.Pro264Ser	No	No	Yes	2.4E-06
1:62601850:A:C	MS (3/5)	c.803A>C	p.Gln268Pro	No	No	Yes	3.7E-05
1:62601864:T:A	MS (4/5)	c.817T>A	p.Tyr273Asn	No	No	Yes	2.4E-06
1:62601867:T:C	MS (4/5)	c.820T>C	p.Cys274Arg	No	No	Yes	5.9E-05
1:62601876:A:C	MS (0/5)	c.829A>C	p.Ile277Leu	No	No	Yes	1.9E-06
1:62601876:A:G	MS (0/5)	c.829A>G	p.Ile277Val	No	No	Yes	1.3E-05
1:62601877:T:C	MS (0/5)	c.830T>C	p.Ile277Thr	No	No	Yes	2.8E-06
1:62601882:G:C	MS (3/5)	c.835G>C	p.Gly279Arg	No	No	Yes	2.4E-05
1:62602285:G:A	MS (1/5)	c.836G>A	p.Gly279Asp	No	No	Yes	1.2E-06
1:62602287:A:T	MS (3/5)	c.838A>T	p.Ser280Cys	No	No	Yes	1.2E-06
1:62602288:G:T	MS (3/5)	c.839G>T	p.Ser280Ile	No	No	Yes	1.2E-06
1:62602289:T:A	MS (0/5)	c.840T>A	p.Ser280Arg	No	No	Yes	4.9E-06
1:62602291:C:A	MS (2/5)	c.842C>A	p.Pro281Gln	No	No	Yes	1.2E-06
1:62602293:T:C	MS (1/5)	c.844T>C	p.Trp282Arg	No	No	Yes	1.5E-04

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62602297:C:T	MS (3/5)	c.848C>T	p.Thr283Ile	No	No	Yes	4.4E-06
1:62602299:T:A	MS (3/5)	c.850T>A	p.Leu284Ile	No	No	Yes	4.4E-06
1:62602309:A:T	MS (2/5)	c.860A>T	p.His287Leu	No	No	Yes	8.8E-06
1:62602312:G:A	MS (4/5)	c.863G>A	p.Arg288Gln	No	No	Yes	2.3E-05
1:62602314:A:T	MS (0/5)	c.865A>T	p.Ile289Leu	No	No	Yes	4.4E-06
1:62602317:G:A	MS (2/5)	c.868G>A	p.Asp290Asn	No	No	Yes	4.9E-06
1:62602335:A:G	MS (3/5)	c.886A>G	p.Asn296Asp	No	No	Yes	2.4E-04
1:62602336:A:G	MS (4/5)	c.887A>G	p.Asn296Ser	No	No	Yes	6.3E-05
1:62602341:A:C	MS (4/5)	c.892A>C	p.Thr298Pro	No	No	Yes	1.2E-06
1:62602349:G:T	MS (1/5)	c.900G>T	p.Glu300Asp	No	No	Yes	1.2E-06
1:62602357:A:C	MS (0/5)	c.908A>C	p.Lys303Thr	No	No	Yes	8.5E-06
1:62602360:A:G	MS (4/5)	c.911A>G	p.Tyr304Cys	No	No	Yes	1.2E-06
1:62602374:C:T	MS (2/5)	c.925C>T	p.Leu309Phe	No	No	Yes	5.9E-05
1:62602375:T:C	MS (4/5)	c.926T>C	p.Leu309Pro	No	No	Yes	1.2E-06
1:62602377:G:A	MS (1/5)	c.928G>A	p.Asp310Asn	No	No	Yes	8.8E-06
1:62602380:G:A	MS (4/5)	c.931G>A	p.Gly311Arg	No	No	Yes	4.4E-06
1:62603998:T:C	MS (1/5)	c.961T>C	p.Tyr321His	No	No	Yes	4.7E-04
1:62604004:A:G	MS (4/5)	c.967A>G	p.Ile323Val	No	No	Yes	9.5E-06
1:62604010:A:C	MS (1/5)	c.973A>C	p.Lys325Gln	No	No	Yes	1.2E-06
1:62604013:C:A	MS (3/5)	c.976C>A	p.Gln326Lys	No	No	Yes	1.0E-05
1:62604019:A:G	MS (0/5)	c.982A>G	p.Asn328Asp	No	No	Yes	1.2E-06
1:62604022:T:C	MS (4/5)	c.985T>C	p.Tyr329His	No	No	Yes	4.4E-06
1:62604032:G:A	MS (3/5)	c.995G>A	p.Arg332Gln	No	No	Yes	4.2E-05
1:62604037:G:A	MS (4/5)	c.1000G>A	p.Glu334Lys	No	No	Yes	2.4E-06
1:62604039:G:T	MS (3/5)	c.1002G>T	p.Glu334Asp	No	No	Yes	1.4E-05
1:62604040:T:A	MS (4/5)	c.1003T>A	p.Leu335Met	No	No	Yes	1.3E-05
1:62604055:G:A	MS (2/5)	c.1018G>A	p.Asp340Asn	No	No	Yes	1.2E-06
1:62604058:A:G	MS (2/5)	c.1021A>G	p.Asn341Asp	No	No	Yes	1.2E-06
1:62604065:A:G	MS (0/5)	c.1028A>G	p.His343Arg	No	No	Yes	9.3E-05

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62604067:T:C	MS (0/5)	c.1030T>C	p.Tyr344His	No	No	Yes	1.2E-06
1:62604071:T:C	MS (0/5)	c.1034T>C	p.Ile345Thr	No	No	Yes	2.9E-06
1:62604082:T:C	MS (2/5)	c.1045T>C	p.Phe349Leu	No	No	Yes	3.6E-06
1:62604085:T:C	MS (0/5)	c.1048T>C	p.Tyr350His	No	No	Yes	1.2E-06
1:62604091:G:A	MS (4/5)	c.1054G>A	p.Gly352Arg	No	No	Yes	4.4E-06
1:62604097:C:T	MS (3/5)	c.1060C>T	p.His354Tyr	No	No	Yes	1.2E-06
1:62604100:G:A	MS (4/5)	c.1063G>A	p.Glu355Lys	No	No	Yes	6.5E-06
1:62604113:C:T	MS (3/5)	c.1076C>T	p.Thr359Met	No	No	Yes	1.2E-06
1:62604118:C:T	MS (4/5)	c.1081C>T	p.His361Tyr	No	No	Yes	6.0E-06
1:62604128:C:G	MS (0/5)	c.1091C>G	p.Ala364Gly	No	No	Yes	2.4E-06
1:62604128:C:T	MS (0/5)	c.1091C>T	p.Ala364Val	No	No	Yes	3.4E-05
1:62604130:A:G	MS (1/5)	c.1093A>G	p.Ile365Val	No	No	Yes	2.4E-06
1:62604131:T:C	MS (1/5)	c.1094T>C	p.Ile365Thr	No	No	Yes	1.2E-06
1:62604141:T:A	MS (3/5)	c.1104T>A	p.Asn368Lys	No	No	Yes	4.4E-06
1:62604142:G:A	MS (0/5)	c.1105G>A	p.Val369Ile	No	No	Yes	1.0E-04
1:62604149:A:C	MS (0/5)	c.1112A>C	p.Asn371Thr	No	No	Yes	2.1E-05
1:62604149:A:G	MS (0/5)	c.1112A>G	p.Asn371Ser	No	No	Yes	5.0E-05
1:62604149:A:T	MS (2/5)	c.1112A>T	p.Asn371Ile	No	No	Yes	2.5E-04
1:62604158:C:T	MS (2/5)	c.1121C>T	p.Pro374Leu	No	No	Yes	1.1E-05
1:62604160:G:A	MS (3/5)	c.1123G>A	p.Glu375Lys	No	No	Yes	2.0E-04
1:62604160:G:C	MS (4/5)	c.1123G>C	p.Glu375Gln	No	No	Yes	1.2E-06
1:62604161:A:C	MS (3/5)	c.1124A>C	p.Glu375Ala	No	No	Yes	4.4E-06
1:62604172:T:G	MS (4/5)	c.1135T>G	p.Leu379Val	No	No	Yes	1.2E-06
1:62604175:G:A	MS (0/5)	c.1138G>A	p.Val380Met	No	No	Yes	9.5E-06
1:62604187:T:C	MS (1/5)	c.1150T>C	p.Trp384Arg	No	No	Yes	3.8E-05
1:62604197:A:G	MS (0/5)	c.1160A>G	p.Lys387Arg	No	No	Yes	4.4E-06
1:62604198:A:C	MS (0/5)	c.1161A>C	p.Lys387Asn	No	No	Yes	4.4E-06
1:62604202:A:C	MS (2/5)	c.1165A>C	p.Lys389Gln	No	No	Yes	5.9E-05
1:62604202:A:G	MS (1/5)	c.1165A>G	p.Lys389Glu	No	No	Yes	1.2E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62604203:A:G	MS (2/5)	c.1166A>G	p.Lys389Arg	No	No	Yes	6.1E-06
1:62604206:G:A	MS (0/5)	c.1169G>A	p.Gly390Glu	No	No	Yes	1.2E-06
1:62604213:C:G	MS (0/5)	c.1176C>G	p.Phe392Leu	No	No	Yes	1.2E-06
1:62604214:A:G	MS (0/5)	c.1177A>G	p.Asn393Asp	No	No	Yes	4.1E-05
1:62604215:A:G	MS (0/5)	c.1178A>G	p.Asn393Ser	No	No	Yes	1.2E-06
1:62604216:C:G	MS (1/5)	c.1179C>G	p.Asn393Lys	No	No	Yes	1.2E-06
1:62604223:G:C	MS (2/5)	c.1186G>C	p.Glu396Gln	No	No	Yes	1.2E-06
1:62604224:A:G	MS (4/5)	c.1187A>G	p.Glu396Gly	No	No	Yes	8.6E-06
1:62604225:G:C	MS (4/5)	c.1188G>C	p.Glu396Asp	No	No	Yes	4.4E-06
1:62604230:A:C	MS (2/5)	c.1193A>C	p.Tyr398Ser	No	No	Yes	4.9E-06
1:62604644:T:C	MS (2/5)	c.1210T>C	p.Trp404Arg	No	No	Yes	4.4E-06
1:62604647:C:T	MS (0/5)	c.1213C>T	p.His405Tyr	No	No	Yes	4.4E-06
1:62604648:A:G	MS (0/5)	c.1214A>G	p.His405Arg	No	No	Yes	1.2E-06
1:62604649:T:G	MS (0/5)	c.1215T>G	p.His405Gln	No	No	Yes	2.4E-04
1:62604652:T:G	MS (0/5)	c.1218T>G	p.Asp406Glu	No	No	Yes	1.8E-05
1:62604653:G:A	MS (0/5)	c.1219G>A	p.Glu407Lys	No	No	Yes	4.4E-06
1:62604653:G:C	MS (0/5)	c.1219G>C	p.Glu407Gln	No	No	Yes	1.2E-06
1:62604654:A:T	MS (0/5)	c.1220A>T	p.Glu407Val	No	No	Yes	4.4E-06
1:62604659:G:A	MS (2/5)	c.1225G>A	p.Gly409Arg	No	No	Yes	1.2E-06
1:62604663:A:C	MS (2/5)	c.1229A>C	p.Glu410Ala	No	No	Yes	6.7E-06
1:62604663:A:G	MS (1/5)	c.1229A>G	p.Glu410Gly	No	No	Yes	5.1E-05
1:62604666:A:G	MS (1/5)	c.1232A>G	p.Asn411Ser	No	No	Yes	1.3E-05
1:62604668:A:C	MS (3/5)	c.1234A>C	p.Asn412His	No	No	Yes	4.9E-06
1:62604705:C:T	MS (0/5)	c.1271C>T	p.Ser424Phe	No	No	Yes	2.4E-06
1:62604707:A:G	MS (3/5)	c.1273A>G	p.Lys425Glu	No	No	Yes	5.9E-05
1:62604713:G:C	MS (1/5)	c.1279G>C	p.Glu427Gln	No	No	Yes	1.5E-05
1:62604716:A:G	MS (3/5)	c.1282A>G	p.Arg428Gly	No	No	Yes	1.2E-06
1:62604723:G:A	MS (1/5)	c.1289G>A	p.Arg430Lys	No	No	Yes	1.2E-04
1:62604723:G:C	MS (4/5)	c.1289G>C	p.Arg430Thr	No	No	Yes	2.8E-05

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62604725:G:A	MS (4/5)	c.1291G>A	p.Gly431Arg	No	No	Yes	4.4E-06
1:62604725:G:C	MS (4/5)	c.1291G>C	p.Gly431Arg	No	No	Yes	1.2E-06
1:62604728:T:A	MS (1/5)	c.1294T>A	p.Leu432Ile	No	No	Yes	8.8E-06
1:62604731:T:A	MS (2/5)	c.1297T>A	p.Ser433Thr	No	No	Yes	1.2E-06
1:62604732:C:A	MS (0/5)	c.1298C>A	p.Ser433Tyr	No	No	Yes	4.8E-06
1:62604732:C:T	MS (0/5)	c.1298C>T	p.Ser433Phe	No	No	Yes	1.5E-05
1:62604743:C:A	MS (1/5)	c.1309C>A	p.Gln437Lys	No	No	Yes	4.4E-06
1:62604744:A:C	MS (1/5)	c.1310A>C	p.Gln437Pro	No	No	Yes	1.8E-05
1:62604749:G:A	MS (2/5)	c.1315G>A	p.Gly439Arg	No	No	Yes	9.7E-06
1:62604750:G:T	MS (4/5)	c.1316G>T	p.Gly439Val	No	No	Yes	2.8E-06
1:62604754:G:T	MS (1/5)	c.1320G>T	p.Arg440Ser	No	No	Yes	5.1E-05
1:62604761:T:C	MS (1/5)	c.1327T>C	p.Ser443Pro	No	No	Yes	1.2E-06
1:62604764:A:T	MS (1/5)	c.1330A>T	p.Ile444Leu	No	No	Yes	9.5E-06
1:62604765:T:C	MS (4/5)	c.1331T>C	p.Ile444Thr	No	No	Yes	1.0E-05
1:62604770:T:C	MS (4/5)	c.1336T>C	p.Ser446Pro	No	No	Yes	1.8E-05
1:62604776:A:G	MS (4/5)	c.1342A>G	p.Lys448Glu	No	No	Yes	4.9E-06
1:62604785:A:G	MS (2/5)	c.1351A>G	p.Ile451Val	No	No	Yes	2.4E-06
1:62604799:T:G	MS (0/5)	c.1365T>G	p.Asp455Glu	No	No	Yes	4.2E-05
1:62604810:T:C	MS (0/5)	c.1376T>C	p.Phe459Ser	No	No	Yes	5.4E-06
1:62604811:T:G	MS (1/5)	c.1377T>G	p.Phe459Leu	No	No	Yes	4.4E-06
1:62604812:G:C	MS (2/5)	c.1378G>C	p.Glu460Gln	No	No	Yes	1.2E-06
1:62604813:A:C	MS (2/5)	c.1379A>C	p.Glu460Ala	No	No	Yes	1.2E-06
1:62597562:T:T A	FS	c.1dupA	p.Met1fs	No	Yes	Yes	2.4E-06
1:62597568:T:G	start_loss	c.2T>G	p.Met1?	No	Yes	Yes	5.1E-05
1:62597608:T:T A	FS	c.43dupA	p.Ile15fs	No	Yes	Yes	2.7E-05

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62597633:A:A G	FS	c.67_68insG	p.Asn23fs	No	Yes	Yes	1.2E-06
1:62597634:A:A T	FS	c.70dupT	p.Ser24fs	No	Yes	Yes	1.2E-06
1:62597670:C:G	SG	c.104C>G	p.Ser35*	No	Yes	Yes	1.2E-06
1:62597673:G:C	MS (5/5)	c.107G>C	p.Arg36Thr	No	Yes	Yes	1.2E-06
1:62597674:A:T	MS (5/5)	c.108A>T	p.Arg36Ser	No	Yes	Yes	4.4E-06
1:62597676:T:C	MS (5/5)	c.110T>C	p.Phe37Ser	No	Yes	Yes	2.4E-06
1:62597676:T:G	MS (5/5)	c.110T>G	p.Phe37Cys	No	Yes	Yes	1.2E-06
1:62597679:C:A	MS (5/5)	c.113C>A	p.Ala38Asp	No	Yes	Yes	2.4E-06
1:62597685:T:C	MS (5/5)	c.119T>C	p.Leu40Ser	No	Yes	Yes	4.4E-06
1:62597690:G:A	MS (5/5)	c.124G>A	p.Asp42Asn	No	Yes	Yes	2.2E-05
1:62597691:A:T	MS (5/5)	c.125A>T	p.Asp42Val	No	Yes	Yes	4.4E-06
1:62597693:G:A	MS (5/5)	c.127G>A	p.Val43Ile	No	Yes	Yes	1.2E-06
1:62597694:T:C	MS (5/5)	c.128T>C	p.Val43Ala	No	Yes	Yes	4.0E-05
1:62597715:T:A	MS (5/5)	c.149T>A	p.Leu50His	No	Yes	Yes	4.4E-06
1:62597717:C:G	MS (5/5)	c.151C>G	p.Leu51Val	No	Yes	Yes	7.4E-05
1:62597723:T:G	MS (5/5)	c.157T>G	p.Leu53Val	No	Yes	Yes	1.2E-06
1:62597727:G:A	MS (5/5)	c.161G>A	p.Gly54Glu	No	Yes	Yes	1.2E-06
1:62597731:TG: T	FS	c.167delG	p.Gly56fs	No	Yes	Yes	2.4E-06
1:62597736:T:G	MS (5/5)	c.170T>G	p.Leu57Arg	No	Yes	Yes	4.4E-06
1:62597742:AC: A	FS	c.177delC	p.Phe60fs	No	Yes	Yes	1.1E-06
1:62597743:CTT :C	FS	c.179_180de ITT	p.Phe60fs	No	Yes	Yes	5.1E-05
1:62597751:A:G	MS (5/5)	c.185A>G	p.His62Arg	No	Yes	Yes	3.8E-06
1:62597754:A:C	MS (5/5)	c.188A>C	p.Lys63Thr	No	Yes	Yes	1.8E-04
1:62597754:A:T	MS (5/5)	c.188A>T	p.Lys63Met	No	Yes	Yes	8.8E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62597757:C:T	MS (5/5)	c.191C>T	p.Thr64Met	No	Yes	Yes	1.9E-06
1:62597759:A:T	SG	c.193A>T	p.Lys65*	No	Yes	Yes	1.2E-06
1:62597774:G:A	MS (5/5)	c.208G>A	p.Asp70Asn	No	Yes	Yes	8.7E-05
1:62597787:A:C	MS (5/5)	c.221A>C	p.Lys74Thr	No	Yes	Yes	1.2E-06
1:62597789:C:T	MS (5/5)	c.223C>T	p.Leu75Phe	No	Yes	Yes	5.9E-05
1:62597801:G:T	MS (5/5)	c.235G>T	p.Asp79Tyr	No	Yes	Yes	4.4E-06
1:62597815:T:G	SG	c.249T>G	p.Tyr83*	No	Yes	Yes	1.2E-06
1:62597823:C:G	MS (5/5)	c.257C>G	p.Ser86Trp	No	Yes	Yes	1.2E-06
1:62597846:G:T	SG	c.280G>T	p.Glu94*	No	Yes	Yes	1.2E-06
1:62597862:T:C	MS (5/5)	c.296T>C	p.Leu99Pro	No	Yes	Yes	4.4E-06
1:62597885:C:T	SG	c.319C>T	p.Gln107*	No	Yes	Yes	4.4E-06
1:62597896:T:G	MS (5/5)	c.330T>G	p.Asn110Lys	No	Yes	Yes	1.2E-06
1:62597901:A:C	MS (5/5)	c.335A>C	p.Glu112Ala	No	Yes	Yes	1.2E-06
1:62597915:T:G	MS (5/5)	c.349T>G	p.Ser117Ala	No	Yes	Yes	4.4E-06
1:62597946:T:C	MS (5/5)	c.380T>C	p.Leu127Pro	No	Yes	Yes	1.2E-06
1:62597954:G:G A	FS	c.394dupA	p.Ile132fs	No	Yes	Yes	2.4E-06
1:62597967:T:A	MS (5/5)	c.401T>A	p.Leu134His	No	Yes	Yes	1.2E-06
1:62597972:CAA :C	FS	c.410_411de IAA	p.Lys137fs	No	Yes	Yes	1.2E-06
1:62598020:C:T	SG	c.454C>T	p.Gln152*	No	Yes	Yes	1.2E-06
1:62598048:TA: T	FS	c.484delA	p.Thr162fs	No	Yes	Yes	1.2E-06
1:62598059:A:G	MS (5/5)	c.493A>G	p.Lys165Glu	No	Yes	Yes	2.4E-04
1:62598059:AA AGT:A	FS	c.494_495+2 delAAGT	p.Lys165fs	No	Yes	Yes	2.4E-05
1:62598700:T:C	MS (5/5)	c.500T>C	p.Phe167Ser	No	Yes	Yes	4.4E-06
1:62598711:C:T	SG	c.511C>T	p.Gln171*	No	Yes	Yes	1.2E-06
1:62598714:G:C	MS (5/5)	c.514G>C	p.Asp172His	No	Yes	Yes	2.7E-05

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62598714:G:T	MS (5/5)	c.514G>T	p.Asp172Tyr	No	Yes	Yes	1.0E-04
1:62598715:A:G	MS (5/5)	c.515A>G	p.Asp172Gly	No	Yes	Yes	1.2E-06
1:62598724:T:C	MS (5/5)	c.524T>C	p.Ile175Thr	No	Yes	Yes	1.2E-05
1:62598762:CAA TT:C	FS	c.565_568de ITTA	p.Leu189fs	No	Yes	Yes	1.2E-06
1:62598787:T:C	MS (5/5)	c.587T>C	p.Ile196Thr	No	Yes	Yes	7.1E-04
1:62598798:GA: G	FS	c.602delA	p.Asn201fs	No	Yes	Yes	5.9E-05
1:62598799:A:G	MS (5/5)	c.599A>G	p.Glu200Gly	No	Yes	Yes	1.2E-06
1:62598807:G:A	SD	c.606+1G>A		No	Yes	Yes	5.1E-05
1:62598807:G:C	SD	c.606+1G>C		No	Yes	Yes	1.2E-06
1:62601080:A:G	SA	c.607-2A>G		No	Yes	Yes	2.4E-06
1:62601198:T:C	SD	c.721+2T>C		No	Yes	Yes	4.4E-06
1:62601783:T:C	MS (5/5)	c.736T>C	p.Cys246Arg	No	Yes	Yes	4.4E-06
1:62601783:T:G	MS (5/5)	c.736T>G	p.Cys246Gly	No	Yes	Yes	1.2E-06
1:62601796:A:G	MS (5/5)	c.749A>G	p.Tyr250Cys	No	Yes	Yes	5.6E-06
1:62601804:G:T	MS (5/5)	c.757G>T	p.Gly253Cys	No	Yes	Yes	2.1E-04
1:62601855:T:C	MS (5/5)	c.808T>C	p.Phe270Leu	No	Yes	Yes	1.2E-06
1:62601861:G:T	MS (5/5)	c.814G>T	p.Val272Phe	No	Yes	Yes	1.2E-06
1:62601868:G:A	MS (5/5)	c.821G>A	p.Cys274Tyr	No	Yes	Yes	7.6E-06
1:62601870:GAT GTT:G	FS	c.826_830de IGTTAT	p.Val276fs	No	Yes	Yes	1.2E-06
1:62602284:G:C	SA	c.836-1G>C		No	Yes	Yes	5.9E-05
1:62602297:C:A	MS (5/5)	c.848C>A	p.Thr283Lys	No	Yes	Yes	3.8E-06
1:62602300:T:C	MS (5/5)	c.851T>C	p.Leu284Ser	No	Yes	Yes	4.8E-06
1:62602305:C:A	MS (5/5)	c.856C>A	p.Gln286Lys	No	Yes	Yes	8.8E-06
1:62602311:C:T	SG	c.862C>T	p.Arg288*	No	Yes	Yes	7.3E-06
1:62602316:AG ATG:A	FS	c.871_874de IGGAT	p.Gly291fs	No	Yes	Yes	1.2E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62602317:G:C	MS (5/5)	c.868G>C	p.Asp290His	No	Yes	Yes	2.7E-05
1:62602323:T:C	MS (5/5)	c.874T>C	p.Ser292Pro	No	Yes	Yes	1.1E-05
1:62602331:C:A	MS (5/5)	c.882C>A	p.Asn294Lys	No	Yes	Yes	5.1E-05
1:62602332:T:C	MS (5/5)	c.883T>C	p.Phe295Leu	No	Yes	Yes	4.4E-06
1:62602332:T:G	MS (5/5)	c.883T>G	p.Phe295Val	No	Yes	Yes	5.1E-05
1:62602337:T:A	MS (5/5)	c.888T>A	p.Asn296Lys	No	Yes	Yes	9.3E-04
1:62602338:GA: G	FS	c.892delA	p.Thr298fs	No	Yes	Yes	1.2E-06
1:62602342:C:T	MS (5/5)	c.893C>T	p.Thr298Met	No	Yes	Yes	1.2E-05
1:62602345:G:A	SG	c.896G>A	p.Trp299*	No	Yes	Yes	5.9E-05
1:62602346:G:A	SG	c.897G>A	p.Trp299*	No	Yes	Yes	1.2E-06
1:62602353:T:C	MS (5/5)	c.904T>C	p.Tyr302His	No	Yes	Yes	4.4E-06
1:62602355:C:A	SG	c.906C>A	p.Tyr302*	No	Yes	Yes	1.2E-06
1:62602373:GC: G	FS	c.925delC	p.Asp310fs	No	Yes	Yes	2.4E-06
1:62602381:G:A	SD	c.931+1G>A		No	Yes	Yes	2.4E-06
1:62603986:C:C T	FS	c.950dupT	p.Glu318fs	No	Yes	Yes	7.1E-04
1:62603993:A:G	MS (5/5)	c.956A>G	p.Lys319Arg	No	Yes	Yes	4.6E-05
1:62603996:T:C	MS (5/5)	c.959T>C	p.Ile320Thr	No	Yes	Yes	1.2E-06
1:62604005:T:C	MS (5/5)	c.968T>C	p.Ile323Thr	No	Yes	Yes	2.4E-06
1:62604017:CTA AT:C	FS	c.983_986de IATTA	p.Asn328fs	No	Yes	Yes	7.3E-06
1:62604024:T:A	SG	c.987T>A	p.Tyr329*	No	Yes	Yes	1.2E-06
1:62604031:C:T	SG	c.994C>T	p.Arg332*	No	Yes	Yes	7.3E-06
1:62604035:T:A	MS (5/5)	c.998T>A	p.Ile333Asn	No	Yes	Yes	1.2E-06
1:62604035:T:G	MS (5/5)	c.998T>G	p.Ile333Ser	No	Yes	Yes	3.1E-04
1:62604037:G:T	SG	c.1000G>T	p.Glu334*	No	Yes	Yes	1.2E-06
1:62604044:A:G	MS (5/5)	c.1007A>G	p.Glu336Gly	No	Yes	Yes	1.2E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62604049:T:C	MS (5/5)	c.1012T>C	p.Trp338Arg	No	Yes	Yes	1.2E-06
1:62604051:G:C	MS (5/5)	c.1014G>C	p.Trp338Cys	No	Yes	Yes	1.2E-06
1:62604083:T:G	MS (5/5)	c.1046T>G	p.Phe349Cys	No	Yes	Yes	1.2E-06
1:62604109:T:C	MS (5/5)	c.1072T>C	p.Tyr358His	No	Yes	Yes	6.1E-06
1:62604160:GA:G	FS	c.1127delA	p.Asn376fs	No	Yes	Yes	1.0E-04
1:62604170:AT:A	FS	c.1136delT	p.Leu379fs	No	Yes	Yes	1.2E-06
1:62604184:A:T	MS (5/5)	c.1147A>T	p.Thr383Ser	No	Yes	Yes	7.3E-05
1:62604185:C:G	MS (5/5)	c.1148C>G	p.Thr383Ser	No	Yes	Yes	9.0E-07
1:62604185:C:T	MS (5/5)	c.1148C>T	p.Thr383Ile	No	Yes	Yes	5.7E-06
1:62604187:TG:T	FS	c.1153delG	p.Asp385fs	No	Yes	Yes	5.1E-05
1:62604190:G:C	MS (5/5)	c.1153G>C	p.Asp385His	No	Yes	Yes	5.9E-05
1:62604190:G:T	MS (5/5)	c.1153G>T	p.Asp385Tyr	No	Yes	Yes	1.2E-06
1:62604218:G:C	MS (5/5)	c.1181G>C	p.Cys394Ser	No	Yes	Yes	1.2E-06
1:62604224:A:A:GG	FS	c.1189_1190 dupGG	p.Tyr398fs	No	Yes	Yes	1.2E-06
1:62604224:AG:A	FS	c.1190delG	p.Gly397fs	No	Yes	Yes	1.3E-05
1:62604233:C:A	SG	c.1196C>A	p.Ser399*	No	Yes	Yes	1.0E-04
1:62604235:G:A	MS (5/5)	c.1198G>A	p.Gly400Arg	No	Yes	Yes	1.2E-06
1:62604639:G:A	SG	c.1205G>A	p.Trp402*	No	Yes	Yes	2.4E-06
1:62604640:G:G:T	FS	c.1207dupT	p.Trp403fs	No	Yes	Yes	1.2E-06
1:62604643:G:C	MS (5/5)	c.1209G>C	p.Trp403Cys	No	Yes	Yes	4.4E-06
1:62604656:T:C	MS (5/5)	c.1222T>C	p.Cys408Arg	No	Yes	Yes	1.1E-05
1:62604669:AC:A	FS	c.1237delC	p.Leu413fs	No	Yes	Yes	1.2E-06

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62604684:A:G	MS (5/5)	c.1250A>G	p.Tyr417Cys	No	Yes	Yes	1.8E-05
1:62604701:A:C	MS (5/5)	c.1267A>C	p.Lys423Gln	No	Yes	Yes	1.2E-06
1:62604726:G:A	MS (5/5)	c.1292G>A	p.Gly431Glu	No	Yes	Yes	3.9E-05
1:62604737:A:G	MS (5/5)	c.1303A>G	p.Lys435Glu	No	Yes	Yes	4.4E-06
1:62604780:T:G	MS (5/5)	c.1346T>G	p.Met449Arg	No	Yes	Yes	1.2E-06
1:62604803:G:T	SG	c.1369G>T	p.Glu457*	No	Yes	Yes	5.9E-05
1:62604806:AG: A	FS	c.1373delG	p.Ser458fs	No	Yes	Yes	1.2E-06
1:62604809:T:T GAATCTG	FS	c.1375_1376 insGAATCTG	p.Phe459fs	No	Yes	Yes	1.2E-06
1:62597855:A:T	SG	c.289A>T	p.Lys97*	Yes	Yes	Yes	3.8E-06
1:62597909:AAT :A	FS	c.346_347de IAT	p.Met116fs	Yes	Yes	Yes	8.6E-06
1:62597921:GA ACTC:G	FS	c.363_367de ICTCAA	p.Asn121fs	Yes	Yes	Yes	5.6E-04
1:62597951:G:T	SG	c.385G>T	p.Glu129*	Yes	Yes	Yes	1.9E-06
1:62597996:CAA CT:C	FS	c.439_442de IAACT	p.Asn147fs	Yes	Yes	Yes	2.1E-04
1:62598753:CA: C	FS	c.555delA	p.Gln185fs	Yes	Yes	Yes	5.7E-06
1:62598771:CA: C	FS	c.573delA	p.Gln191fs	Yes	Yes	Yes	7.2E-06
1:62598774:C:T	SG	c.574C>T	p.Gln192*	Yes	Yes	Yes	1.9E-06
1:62598774:CA: C	FS	c.575delA	p.Gln192fs	Yes	Yes	Yes	2.6E-05
1:62601115:AT: A	FS	c.643delT	p.Ser215fs	Yes	Yes	Yes	2.7E-05
1:62601168:GA: G	FS	c.695delA	p.Asn232fs	Yes	Yes	Yes	1.4E-05

Genomic coordinate for the genetic variant, C:P:R:A	Predicted consequ. of variant	Coding DNA change	Protein amino acid change	Variant classified as pLOF	Variant classified as pLOF or 5/5 missense	Variant classified as pLOF or any missense	AAF
1:62601197:G:A	SD	c.721+1G>A		Yes	Yes	Yes	1.2E-06
1:62601197:G:T	SD	c.721+1G>T		Yes	Yes	Yes	4.4E-06
1:62601880:C:A	SG	c.833C>A	p.Ser278*	Yes	Yes	Yes	1.3E-05
1:62601880:C:G	SG	c.833C>G	p.Ser278*	Yes	Yes	Yes	5.7E-06
1:62602297:C:C A	FS	c.849dupA	p.Leu284fs	Yes	Yes	Yes	4.4E-06
1:62602381:G:G T	SD	c.931+1_931 +2insT		Yes	Yes	Yes	1.6E-05
1:62602382:T:C	SD	c.931+2T>C		Yes	Yes	Yes	4.4E-06
1:62604236:G:T	SD	c.1198+1G> T		Yes	Yes	Yes	5.6E-06
1:62604645:G:A	SG	c.1211G>A	p.Trp404*	Yes	Yes	Yes	3.1E-05
1:62604762:C:C T	FS	c.1329dupT	p.Ile444fs	Yes	Yes	Yes	9.5E-06

MS = missense; FS = frameshift; SG = stop_gained; SD = splice_donor; SA = splice_acceptor; C:P:R:A indicates the genomic coordinates of the genetic variant including chromosome (C), physical genomic position in base pairs (P), reference allele (R) and alternative allele (A) relative to build 38 of the Human Genome sequence by the Human Genome Reference Consortium. Coding DNA and protein changes follow the Human Genome Variation Society nomenclature and refer to *ANGPTL3* transcript (ENST00000371129 annotated in the in the Ensembl database (URL: world wide web at <http://useast.ensembl.org/index.html>)). Missense (0/5), Missense (1/5), Missense (2/5), Missense (3/5), Missense (4/5), Missense (5/5) indicate missense variants predicted to be damaging by 0, 1, 2, 3, 4, or 5 *in silico* algorithms, respectively. AAF indicates the alternative allele frequency. pLOF indicates predicted loss of function variant. Variant classified as indicates whether the variant was included in gene burden grouping specified.

Participating cohorts

Genetic association studies were performed in the United Kingdom (UK) Biobank (UKB) cohort (Sudlow et al., PLoS Med., 2015, 12, e1001779) and the DiscoverEHR cohort from the Geisinger Health System (GHS) MyCode Community Health Initiative (Carey et al., Genet. Med., 2016, 18, 906-13). The UKB is a population-based cohort study of people aged between 40 and 69 years recruited through 22 testing centers in the UK between 2006-2010. Over 430,000 participants of varying ancestries from UKB with available whole-exome sequencing and clinical phenotype data were included. The GHS MyCode study Community Health Initiative is a health system-based cohort of patients from Central and Eastern Pennsylvania (USA) recruited in 2007-2019. Over 130,000 European ancestry participants from GHS with available whole-exome sequencing and clinical phenotype data were included.

Phenotype definitions

eGFR was calculated from clinical laboratory measurements for creatinine extracted from electronic health records (EHRs) of participants from GHS. Median values were calculated for all participants with two or more measurements. In UKB, $eGFR_{creatinine}$ was calculated from creatinine measured on a Beckman Coulter AU5800 clinical chemistry analyzer and $eGFR_{cystatin}$ was calculated from cystatin measured by immunoturbidimetric analysis on a Siemens Advia 1800 clinical chemistry analyzer; both creatinine and cystatin were measured at the baseline visit of the study. In UKB, urine albumin:creatinine ratio (UACR) was derived from creatinine measured by enzymatic analysis and albumin measured by immunoturbidimetric analysis on a Beckman Coulter AU5400 clinical chemistry analyzer on spot urine measured at the baseline visit of the study. Prior to genetic association analysis, continuous phenotype values were transformed by the inverse standard normal function, applied within each ancestry group and separately in men and women. Kidney disease outcomes were defined according to the International Classification of Diseases Tenth Revision (ICD-10) and read codes stored in EHRs. Self-reported disease status was used when available. Office of Population Censuses and Surveys Classification of Interventions and Procedures version 4 (OPCS4) codes were used for medical procedures in UKB.

Individuals with kidney diseases were identified as described in Table 5, combining EHR records, self-reports and eGFR and UACR measurements. Participants were excluded from the control population for all kidney disease outcomes if they met any of the following criteria:

1. ICD10: N00,N01,N02,N03,N04,N05,N06,N07,N08,N10,N11,N12,N13,N14,N15, N16,N17,N18,N19,N20,N21,N22,N23,N25,N26,N27,N28,N29,Q60,Q61,Q63,C64,R944,I12,

I13,T861,Z940,O904,O084,T824,Y841,Z490,Z491,Z492,Z992

2. eGFR < 90 ml/min

3. Self-reported illness code (UKB-specific): 1192,1193,1194,1197,1519,1520,1607,1608,1609,1427,1405

5 4. Self-reported operation code (UKB-specific): 1195,1197,1476,1487,1580,1581,1582,1618

5. OPCS4 operation code (UKB-specific): M01,M02,M03,M04,M05,M061,M068,M069,M07,M08,M09,M10,M111,M112,M131,M132,M133,M137,M14,M164,L746,M172,M174,M178,M179,X401,X402,X403,X404,X405,X406,X408,X409,X411,X412,X418,X419,X421,X428,X429,M012,M013,M014,M015,M018,M019,M084

6. Source of end-stage renal disease report code (UKB-specific): 0,1,2

7. Urinary albumin:creatinine ratio > 10 mg/g (UKB-specific)

8. EHR code for kidney transplant or dialysis in billing procedures or surgery (GHS-specific).

15 Table 5: Definitions of kidney disease and outcomes in UKB and GHS cohort

Kidney disease outcome	Case definition Participants were included as a case if they met any of the criteria specific to each disease
Microalbuminuria	UKB.UACR > 30 mg/g
Any kidney disease	ICD10: N00,N01,N02,N03,N04,N05,N06,N07,N08,N10,N11,N12,N13,N14,N15,N16,N17,N18,N19,N25,N26,N27,N28,N29,Q60,Q61,Q63,C64,R944,I12,I13,T861,Z940,O904,O084,T824,Y841,Z490,Z491,Z492,Z992 UKB.f.20002: 1192,1193,1194,1519,1520,1607,1608,1609,1427,1405 UKB.f.20004: 1195,1476,1487,1580,1581,1582,1618 UKB.OPCS4: M01,M02,M03,M04,M05,M068,M069,M078,M079,M08,M10,M111,M112,M131,M132,M133,M137,M164,L746,M172,M174,M178,M17

	9,X401,X402,X403,X404,X405,X406,X408,X409,X411,X412,X418,X419,X421,X428,X429,M012,M013,M014,M015,M018,M019,M084 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant or dialysis in billing procedures or surgery eGFR < 60 ml/min UKB.UACR > 30 mg/g
Chronic kidney disease, any	ICD10: N18,Q611,Q612,Q613,Q615,I12,I13,T861,Z940,Z492 UKB.f.20002: 1193,1607,1427 UKB.f.20004: 1195,1476,1580,1581,1582 UKB.OPSC4: L746,M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 60 ml/min UKB.UACR > 300 mg/g
Chronic kidney disease, any	ICD10: N18,Q611,Q612,Q613,Q615,I12,I13,T861,Z940,Z492 UKB.f.20002: 1193,1607,1427

including microalbuminuria	UKB.f.20004: 1195,1476,1580,1581,1582 UKB.OPSC4: L746,M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 60 ml/min UKB.UACR > 30 mg/g
CKD stage 3+	ICD10: N183,N184,N185,N180,N186,T861,Z492,Z940,N165 UKB.f.20002: 1193 UKB.f.20004: 1476,1195,1580,1581,1582 UKB.OPSC4: M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 60 ml/min
CKD stage 5+	ICD10: N185,N180,N186,T861,Z492,Z940,N165

	UKB.f.20002: 1193 UKB.f.20004: 1476,1195,1580,1581,1582 UKB.OPSC4: M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 15 ml/min
Renal replacement therapy	ICD10: T824,T861,Y841,Z490,Z491,Z492,Z940,Z992 UKB.f.20002: 1193 UKB.f.20004: 1195,1476,1580,1581,1582 UKB.OPSC4: L746,M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X401,X402,X403,X404,X405,X406,X408,X409,X411,X412,X418,X419,X421,X428,X429 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant or dialysis in billing procedures or surgery
End stage renal disease	ICD10: N180,N186,T861,Z492,Z940,N165 UKB.f.20002: 1193

	UKB.f.20004: 1476,1195,1580,1581,1582 UKB.OPSC4: M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery
--	---

ICD10 indicates the 10th revision of the International Statistical Classification of Diseases and Related Health Problems; UKB.OPSC4 indicates Office of Population Censuses and Surveys (OPCS) Classification of Interventions and Procedures version 4 as used in the UK Biobank (UKB); UKB.f.20002 indicates self-reported non-cancer illness codes as used in UKB; UKB.f.20004 indicates self-reported medical procedures as used in UKB; UKB.f.42027 indicates source of end-stage renal disease code as defined by central working group for UKB; GHS.EHR indicates billing procedures or surgeries in Geisinger Health System (GHS) relevant to disease definition; UKB.UACR indicates urine albumin:creatinine ratio measured in UKB.

10 *Genotype data*

High coverage whole exome sequencing was performed as previously described (Science, 2016, 354:aaf6814; and Nature, 2020, 586, 749-756) and as summarized below. NimbleGen probes (VCRome; for part of the GHS cohort) or a modified version of the xGen design available from Integrated DNA Technologies (IDT; for the rest of GHS and other cohorts) were used for target sequence capture of the exome. A unique 6 base pair (bp) barcode (VCRome) or 10 bp barcode (IDT) was added to each DNA fragment during library preparation to facilitate multiplexed exome capture and sequencing. Equal amounts of sample were pooled prior to exome capture. Sequencing was performed using 75 bp paired-end reads on Illumina v4 HiSeq 2500 (for part of the GHS cohort) or NovaSeq (for the rest of GHS and other cohorts) instruments. Sequencing had a coverage depth (i.e., number of sequence-reads covering each nucleotide in the target areas of the genome) sufficient to provide greater than 20x coverage over 85% of targeted bases in 96% of VCRome samples and 20x coverage over 90% of targeted

bases in 99% of IDT samples. Data processing steps included sample de-multiplexing using Illumina software, alignment to the GRCh38 Human Genome reference sequence including generation of binary alignment and mapping files (BAM), processing of BAM files (e.g., marking of duplicate reads and other read mapping evaluations). Variant calling was performed using the GLNexus system (DOI: 10.1101/343970). Variant mapping and annotation were based on the GRCh38 Human Genome reference sequence and Ensembl v85 gene definitions using the snpEff software. The snpEff predictions that involve protein-coding transcripts with an annotated start and stop were then combined into a single functional impact prediction by selecting the most deleterious functional effect class for each gene. The hierarchy (from most to least deleterious) for these annotations was frameshift, stop-gain, stop-loss, splice acceptor, splice donor, stop-lost, in-frame indel, missense, other annotations. Predicted LOF genetic variants included: a) insertions or deletions resulting in a frameshift, b) insertions, deletions or single nucleotide variants resulting in the introduction of a premature stop codon or in the loss of the transcription start site or stop site, and c) variants in donor or acceptor splice sites.

Missense variants were classified for likely functional impact according to the number of *in silico* prediction algorithms that predicted deleteriousness using SIFT (Adzhubei et al., Nat. Methods, 2010, 7, 248-9) and Polyphen2_HVAR (Adzhubei et al., Nat. Methods, 2010, 7, 248-9), LRT (Chun et al., Genome Res., 2009, 19, 1553-61) and MutationTaster (Schwarz et al., Nat. Methods, 2010, 7, 575-6). For each gene, the alternative allele frequency (AAF) and functional annotation of each variant determined inclusion into these 7 gene burden exposures: 1) pLOF variants with AAF < 1%; 2) pLOF or missense variants predicted deleterious by 5/5 algorithms with AAF < 1%; 3) pLOF or missense variants predicted deleterious by 5/5 algorithms with AAF < 0.1%; 4) pLOF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 1%; 5) pLOF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 0.1%; 6) pLOF or any missense with AAF < 1%; 7) pLOF or any missense variants with AAF < 0.1%.

Association analysis of gene burden of rare pLOF and missense variations

The burden of rare predicted loss-of-function or missense variants in a given gene and phenotype were examined for association by fitting a linear (for quantitative traits) or firth bias-corrected logistic (for binary traits) regression model adjusted for a polygenic score that approximates a genomic kinship matrix using REGENIE (Mbatchou et al., Nat. Genetics, 2020, 53, 1097–1103). Analyses were stratified by ancestry and adjusted for age, age², sex, age-by-sex

and age²-by-sex interaction terms, experimental batch-related covariates, 10 common variant-derived principal components, and 20 rare variant-derived principal components. Results across cohorts for each variant-phenotype association were combined using fixed effects inverse variance weighted meta-analysis. In gene burden tests, all individuals are labeled as heterozygotes if they carry one or more qualifying rare variant (as described above based on frequency and functional annotation) and as homozygotes if they carry any qualifying variant in the homozygous state. This “composite genotype” is then used to test for association.

Example 3: Association Between Loss-of-Function of *ANGPTL3* and Improved Kidney Function is Unique Among Lipid Genes

Loss-of-function genetic variation in *ANGPTL3* is known to associate with decreased circulating LDL cholesterol and triglyceride levels (Romeo et al., J. Clin. Invest., 2009, 119, 70-79; and Musunuru et al., N. Engl. J. Med., 2010, 363, 2220-2227), raising the question of whether the association between loss-of-function genetic variation in *ANGPTL3* and improved kidney function is a consequence of lipid lowering. To address this question, we examined whether genetic variation in other known lipid-regulating genes was also associated with improved kidney function.

Low-frequency coding variants in *ANGPTL4*, *PCSK9*, and *LPL* were strongly associated with decreased circulating triglycerides (*ANGPTL4* p.Glu40Lys, *LPL* p.Ser474*) or LDL cholesterol (*PCSK9* p.Arg46Leu), (see Table 5). However, no significant increases in eGFR were observed with these genetic variants (Table 6), as would be expected if the increase in eGFR was secondary to decreased lipids.

Table 5. Associations with decreased triglyceride or LDL cholesterol levels for low-frequency coding variants in *ANGPTL4*, *PCSK9*, and *LPL* genes

Genetic exposure (Gene, amino acid variation)	Outcome	Effect per allele [95%CI]	P	Genotype counts, Ref / Het / Hom	AAF
---	---------	------------------------------	---	--	-----

rs116843064 (ANGPTL4, p.Glu40Lys)	Triglyceride s	-0.24 [-0.25,-0.22] SD, -20.68 [-21.82,-19.54] mg/dL	6.3E -277	503,591 / 19,287 / 196	0.018 8
rs116843064 (ANGPTL4, p.Glu40Lys)	LDL cholesterol	0.03 [0.02,0.05] SD, 1.25 [0.74,1.76] mg/dL	1.6E -06	458,414 / 17,733 / 189	0.019 0
rs11591147 (PCSK9, p.Arg46Leu)	Triglyceride s	-0.01 [-0.02,0.01] SD, -0.60 [-1.82,0.62] mg/dL	0.33	506,140 / 16,844 / 148	0.016 4
rs11591147 (PCSK9, p.Arg46Leu)	LDL cholesterol	-0.37 [-0.39,-0.36] SD, -13.57 [-14.11,-13.03] mg/dL	3.0E -528	461,013 / 15,244 / 136	0.016 3
rs328 (LPL, p.Ser474Ter)	Triglyceride s	-0.20 [-0.20,-0.19] SD, -17.17 [-17.69,-16.65] mg/dL	8.0E -920	425,377 / 94,558 / 5,298	0.100
rs328 (LPL, p.Ser474Ter)	LDL cholesterol	0.02 [0.02,0.03] SD, 0.89 [0.66,1.12] mg/dL	1.1E -14	386,450 / 86,930 / 4,878	0.101

Abbreviations: CI, confidence interval; SD, standard deviation; mg/dL, milligrams per deciliter; AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of genetic exposure variant; Hom, homozygous carriers of genetic exposure variant; LDL, low-density lipoprotein; Glu, glutamate; Lys, lysine; Arg, arginine; Leu, leucine; Ser, serine; Ter, termination codon.

Table 6. Lack of associations with increased eGFR levels for low-frequency coding variants in

ANGPTL4, *PCSK9*, and *LPL* genes

Genetic exposure (Gene, amino acid variation)	Outcome	Effect per allele [95%CI]	P	Genotype counts, Ref / Het / Hom	AAF
---	---------	------------------------------	---	---	-----

rs116843064 (ANGPTL4, p.Glu40Lys)	eGFR _{creatinin} e	-0.00 [-0.01,0.01] SD, -0.03 [-0.22,0.16] mL/min	0.75	523,796 / 20,195 / 200	0.0189 2
rs11591147 (PCSK9, p.Arg46Leu)	eGFR _{creatinin} e	-0.03 [-0.04,-0.02] SD, -0.52 [-0.72,-0.31] mL/min	8.0E -07	526,577 / 17,489 / 155	0.0163 5
rs328 (LPL, p.Ser474Ter)	eGFR _{creatinin} e	-0.00 [-0.01,0.00] SD, -0.06 [-0.15,0.02] mL/min	0.15	442,385 / 98,410 / 5,526	0.1001 8

Abbreviations: CI, confidence interval; SD, standard deviation; mL/min, milliliters per minute; AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of genetic exposure variant; Hom, homozygous carriers of genetic exposure variant; eGFR_{creatinine}, estimated glomerular filtration rate calculated using serum creatinine; Glu, glutamate; Lys, lysine; Arg, arginine; Leu, leucine; Ser, serine; Ter, termination codon.

Low-frequency coding variants with significant effects on lipids exist in only a subset of lipid-regulating genes. Therefore, to more comprehensively examine whether coding variation in lipid-regulating genes associated reduced lipids with improved kidney function, we also examined associations between the burden of rare coding genetic variants in additional lipid-regulating genes and kidney function. The burden of rare coding genetic variants, either pLOF alone or pLOF and missense variants combined, in *ANGPTL3*, *ANGPTL4*, *ANGPTL8*, or *PCSK9* was associated with decreased triglyceride or LDL cholesterol levels, whereas the burden of rare coding genetic variants in *LPL* or *LDLR* was associated with increased triglyceride or LDL cholesterol levels (Table 7), consistent with their known roles in lipid biology.

Table 7. Associations with triglyceride or LDL cholesterol levels for the burden of rare pLOF or missense variants in lipid-regulating genes

Genetic exposure	Outcome	Effect per allele [95%CI]	P	Genotype counts, Ref / Het / Hom	AAF
------------------	---------	------------------------------	---	--	-----

ANGPTL3 pLOF; AAF < 1%	Triglyceride s	-0.64 [-0.69,-0.59] SD, -55.85 [-60.48,- 51.22] mg/dL	1.3E- 123	524,069 / 1,168 / 1	0.0011 1
ANGPTL3 pLOF; AAF < 1%	LDL cholesterol	-0.24 [-0.29,-0.18] SD, -8.66 [-10.68,-6.63] mg/dL	5.5E- 17	477,170 / 1,092 / 0	0.0011 4
ANGPTL3 pLOF or missense (5/5); AAF < 0.1%	Triglyceride s	-0.55 [-0.59,-0.51] SD, -48.09 [-51.39,- 44.80] mg/dL	4.4E- 180	522,914 / 2,321 / 3	0.0022 2
ANGPTL3 pLOF or missense (5/5); AAF < 0.1%	LDL cholesterol	-0.20 [-0.24,-0.16] SD, -7.17 [-8.62,-5.73] mg/dL	2.1E- 22	476,101 / 2,160 / 1	0.0022 6
ANGPTL4 pLOF; AAF < 1%	Triglyceride s	-0.39 [-0.45,-0.33] SD, -33.95 [-39.54,- 28.36] mg/dL	1.1E- 32	523,846 / 805 / 0	0.0007 7
ANGPTL4 pLOF; AAF < 1%	LDL cholesterol	0.05 [-0.02,0.11] SD, 1.65 [-0.83,4.12] mg/dL	0.19	476,987 / 762 / 0	0.0008
ANGPTL4 pLOF or missense (5/5); AAF < 1%	Triglyceride s	-0.35 [-0.39,-0.30] SD, -30.13 [-34.01,- 26.24] mg/dL	3.4E- 52	522,979 / 1,672 / 0	0.0015 9
ANGPTL4 pLOF or missense (5/5); AAF < 1%	LDL cholesterol	0.03 [-0.01,0.08] SD, 1.20 [-0.53,2.93] mg/dL	0.17	476,190 / 1,559 / 0	0.0016 3

ANGPTL8 pLOF; AAF < 1%	Triglyceride s	-0.35 [-0.41,-0.29] SD, -30.34 [-35.49,- 25.18] mg/dL	8.8E- 31	524,294 / 942 / 2	0.0009
ANGPTL8 pLOF; AAF < 1%	LDL cholesterol	-0.02 [-0.08,0.04] SD, -0.76 [-3.07,1.55] mg/dL	0.52	477,390 / 871 / 1	0.0009 1
ANGPTL8 pLOF or missense (5/5); AAF < 0.1%	Triglyceride s	-0.38 [-0.44,-0.31] SD, -32.76 [-38.76,- 26.77] mg/dL	9.1E- 27	524,544 / 692 / 2	0.0006 6
ANGPTL8 pLOF or missense (5/5); AAF < 0.1%	LDL cholesterol	-0.02 [-0.09,0.06] SD, -0.69 [-3.42,2.04] mg/dL	0.62	477,641 / 620 / 1	0.0006 5
LPL pLOF; AAF < 1%	Triglyceride s	0.72 [0.57,0.86] SD, 62.58 [49.95,75.21] mg/dL	2.7E- 22	524,492 / 159 / 0	0.0001 5
LPL pLOF; AAF < 1%	LDL cholesterol	-0.18 [-0.33,-0.03] SD, -6.56 [-12.12,-1.01] mg/dL	0.021	477,606 / 143 / 0	0.0001 5
LPL pLOF or missense (5/5); AAF < 0.1%	Triglyceride s	0.40 [0.35,0.46] SD, 35.16 [30.16,40.16] mg/dL	3.6E- 43	523,634 / 1,017 / 0	0.0009 7
LPL pLOF or missense (5/5); AAF < 0.1%	LDL cholesterol	-0.07 [-0.13,-0.01] SD, -2.62 [-4.83,-0.41] mg/dL	0.02	476,841 / 908 / 0	0.0009 5

LDLR pLOF; AAF < 1%	Triglyceride s	-0.12 [-0.20,-0.03] SD, -10.18 [-17.86,-2.50] mg/dL	0.009 4	524,225 / 426 / 0	0.0004 1
LDLR pLOF; AAF < 1%	LDL cholesterol	0.28 [0.19,0.38] SD, 10.28 [6.82,13.74] mg/dL	5.5E- 09	477,359 / 390 / 0	0.0004 1
LDLR pLOF or missense (5/5); AAF < 0.1%	Triglyceride s	-0.05 [-0.09,-0.02] SD, -4.70 [-7.91,-1.49] mg/dL	0.004 1	522,786 / 2,451 / 1	0.0023 4
LDLR pLOF or missense (5/5); AAF < 0.1%	LDL cholesterol	0.49 [0.45,0.53] SD, 17.84 [16.39,19.28] mg/dL	1.6E- 129	476,015 / 2,247 / 0	0.0023 5
PCSK9 pLOF; AAF < 1%	Triglyceride s	-0.13 [-0.19,-0.07] SD, -11.52 [-16.63,-6.42] mg/dL	9.7E- 06	524,266 / 971 / 1	0.0009 3
PCSK9 pLOF; AAF < 1%	LDL cholesterol	-0.94 [-1.00,-0.88] SD, -34.13 [-36.38,- 31.87] mg/dL	1.2E- 193	477,378 / 883 / 1	0.0009 3
PCSK9 pLOF or missense (5/5); AAF < 0.1%	Triglyceride s	-0.07 [-0.12,-0.03] SD, -6.46 [-10.12,-2.81] mg/dL	5.3E- 04	523,346 / 1,889 / 3	0.0018
PCSK9 pLOF or missense (5/5); AAF < 0.1%	LDL cholesterol	-0.70 [-0.75,-0.66] SD, -25.53 [-27.15,- 23.92] mg/dL	9.9E- 212	476,530 / 1,729 / 3	0.0018 1

Abbreviations: CI, confidence interval; SD, standard deviation; mg/dL, milligrams per deciliter; AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of rare coding variant (defined in 'Genetic exposure'); Hom, homozygous carrier of rare coding variant (defined in 'Genetic exposure'); pLOF, predicted loss of function; missense (5/5), missense variant predicted to be deleterious by 5 out of 5 in silico prediction algorithms; LDL, low-density lipoprotein

However, when examining effects on kidney function, only the burden of rare coding genetic variants in *ANGPTL3* showed a significant effect on kidney function directionally concordant with its effects on lipids (that is, decreased triglycerides or LDL associated with increased eGFR, or increased triglycerides or LDL associated with decreased eGFR, see Table 8). Therefore, the association between loss-of-function genetic variation in *ANGPTL3* and improved kidney function reflects a unique role of *ANGPTL3* function as opposed to generic lipid-lowering.

Table 8. The burden of rare pLOF or missense variants in *ANGPTL3*, but not *ANGPTL4*, *ANGPTL8*, *LPL*, *PCSK9*, or *LDLR* show significant, inverse direction of effect on lipids (see Table 7) and eGFR

Genetic exposure	Outcome	Expected direction of effect	Observed effect per allele	P	Genotype counts, Ref / Het / Hom	AAF
ANGPTL3 pLOF; AAF < 1%	eGFR _{creatinine}	Increase	0.06 [0.02,0.11] SD, 1.03 [0.26,1.79] mL/min	0.0084	545,101 / 1,225 / 1	0.00112
ANGPTL3 pLOF or deleterious missense (5/5); AAF < 0.1%	eGFR _{creatinine}	Increase	0.05 [0.02,0.08] SD, 0.86 [0.32,1.4] mL/min	0.0019	543,886 / 2,437 / 4	0.00224
ANGPTL4 pLOF; AAF < 1%	eGFR _{creatinine}	Increase	0.01 [-0.05,0.06] SD,	0.76	544,886 / 854 / 0	0.00078

			0.14 [- 0.76,1.05] mL/min			
ANGPTL4 pLOF or deleterious missense (5/5); AAF < 1%	eGFR _{creatinine}	Increase	0.01 [- 0.03,0.04] SD, 0.12 [- 0.51,0.76] mL/min	0.7	543,979 / 1,761 / 0	0.0016 1
ANGPTL8 pLOF; AAF < 1%	eGFR _{creatinine}	Increase	-0.06 [-0.11,- 0.02] SD, -1.1 [-1.91,- 0.28] mL/min	0.008 6	545,312 / 1,013 / 2	0.0009 3
ANGPTL8 pLOF or deleterious missense (5/5); AAF < 0.1%	eGFR _{creatinine}	Increase	-0.06 [- 0.12,0.00] SD, -1.03 [-2.06,0] mL/min	0.051	545,620 / 705 / 2	0.0006 5
LPL pLOF; AAF < 1%	eGFR _{creatinine}	Decrease	0.11 [- 0.02,0.24] SD, 1.82 [- 0.34,3.98] mL/min	0.099	545,578 / 162 / 0	0.0001 5
LPL pLOF or deleterious missense (5/5); AAF < 0.1%	eGFR _{creatinine}	Decrease	0.05 [0.00,0.10] SD, 0.82 [0.02,1.62] mL/min	0.045	544,648 / 1,089 / 3	0.001
LDLR pLOF; AAF < 1%	eGFR _{creatinine}	Decrease	-0.02 [- 0.10,0.05] SD, -0.36 [- 1.62,0.91] mL/min	0.58	545,299 / 441 / 0	0.0004

LDLR pLOF or deleterious missense (5/5); AAF < 0.1%	eGFR _{creatinine}	Decrease	-0.00 [-0.03,0.03] SD, -0.06 [-0.59,0.48] mL/min	0.83	543,794 / 2,532 / 1	0.0023 / 2
PCSK9 pLOF; AAF < 1%	eGFR _{creatinine}	Increase	-0.05 [-0.10,0.00] SD, -0.83 [-1.71,0.06] mL/min	0.066	545,344 / 982 / 1	0.0009
PCSK9 pLOF or deleterious missense (5/5); AAF < 0.1%	eGFR _{creatinine}	Increase	-0.03 [-0.07,0.01] SD, -0.51 [-1.12,0.11] mL/min	0.11	544,376 / 1,948 / 3	0.0017 / 9

Abbreviations: CI, confidence interval; SD, standard deviation; mL/min, milliliters per minute; AAF, alternative allele frequency; Ref, homozygous reference genotype; Het, heterozygous carrier of rare coding variant (defined in 'Genetic exposure'); Hom, homozygous carrier of rare coding variant (defined in 'Genetic exposure'); pLOF, predicted loss of function; missense (5/5), missense variant predicted to be deleterious by 5 out of 5 in silico prediction algorithms; eGFR_{creatinine}, estimated glomerular filtration rate calculated using serum creatinine; Expected direction of effect, expected direction of genetic exposure effect on eGFR_{creatinine}, given the most significant (lowest p-value) effect of genetic exposure on LDL (low-density lipoprotein) cholesterol or triglycerides from Table 7 (that is, decreased LDL cholesterol or triglycerides expected to increase eGFR_{creatinine}, whereas increased LDL cholesterol or triglycerides expected to decreased eGFR_{creatinine}).

Example 4: Circulating ANGPTL3 Levels were Positively Associated with Risk of Newly-Onset Chronic Kidney Disease

We assessed the relationship between circulating plasma ANGPTL3 levels and kidney function in 38,043 individuals without evidence of kidney disease or dysfunction. At the time of

measurement, plasma ANGPTL3 was weakly correlated with LDL cholesterol (Partial correlation coefficient=0.21), high density lipoprotein (HDL) cholesterol (0.16), triglycerides (0.12), cystatin-C (0.19), creatinine (0.03), and eGFR_{creatinine} (-0.04), after adjusting for age-at-baseline, sex, and body-mass index.

5 Circulating levels of ANGPTL3 were also positively associated with higher risk of newly-onset CKD (Figure 1), after stratification for sex and study center, and adjustment for age-at-baseline, smoking (current-smoker, ex-smoker, and never-smoker), alcohol consumption (current-drinker, ex-drinker, and never-drinker), history of diabetes (Yes vs. No), HDL cholesterol, LDL cholesterol, eGFR_{creatinine}. Compared to participants who were in the top
10 quartile of the ANGPTL3 levels, the adjusted hazard ratio (HR) for those who were in the bottom quartile was 0.57 (95% CI: 0.45 – 0.71; p=1.19E-6). Results remained similar after further adjustment for baseline levels of triglycerides, and hemoglobin A1c.

15

Table 9: adjusted associations of circulating ANGPTL3 levels and risk of newly-onset chronic kidney disease in UK Biobank

Quartiles	No. of events	No. of participants	ANGPTL3 (log2-transformed)	ANGPTL3, NPX units	Hazard ratios (95% CI)
Q1	184	7609	-0.23	0.85	0.57 (0.48 - 0.67)
Q2	197	7609	0.15	1.11	0.72 (0.63 - 0.84)
Q3	165	7608	0.37	1.29	0.69 (0.59 - 0.80)
Q4	178	7609	0.59	1.51	0.72 (0.62 - 0.84)
Q5	253	7608	0.97	1.96	Reference

20 Analyses were conducted among individuals with prior history of physician-diagnosed CKD, or abnormal eGFR_{creatinine} (defined here as: eGFR <60 mL/min/1.73m² or eGFR >110 mL/min/1.73m²) at recruitment, and those with missing data on covariates (defined here as: systolic blood pressure, HDL cholesterol, LDL cholesterol,, smoking status, alcohol consumption status, history of diabetes at baseline, and eGFR_{creatinine}). Hazard ratios and the corresponding
25 95% confidence intervals were estimated using Cox regression, stratified by sex and study

center, adjusted for age-at-baseline, smoking status, alcohol consumption, history of diabetes, HDL-C, LDL-C, and baseline levels of eGFR_{creatinine}. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR_{creatinine}, estimated glomerular filtration rate calculated from serum creatinine, NPX, Normalized Protein eXpression relative quantification units.

5 *Participating cohorts*

Genetic association studies were performed in the United Kingdom (UK) Biobank (UKB) cohort (Sudlow et al., PLoS Med., 2015, 12, e1001779) and the DiscoverEHR cohort from the Geisinger Health System (GHS) MyCode Community Health Initiative (Carey et al., Genet. Med., 2016, 18, 906-13). The UKB is a population-based cohort study of people aged between 40 and
10 69 years recruited through 22 testing centers in the UK between 2006-2010. Over 430,000 participants of varying ancestries from UKB with available whole-exome sequencing and clinical phenotype data were included. Among a subset of the entire UK Biobank prospective study, proteomic profiling was conducted on blood plasma samples collected from n=54,306 individuals at baseline. The GHS MyCode study Community Health Initiative is a health system-
15 based cohort of patients from Central and Eastern Pennsylvania (USA) recruited in 2007-2019. Over 130,000 European ancestry participants from GHS with available whole-exome sequencing and clinical phenotype data were included.

Phenotype definitions

eGFR was calculated from clinical laboratory measurements for creatinine extracted
20 from electronic health records (EHRs) of participants from GHS. Median values were calculated for all participants with two or more measurements. In UKB, eGFR_{creatinine} was calculated from creatinine measured on a Beckman Coulter AU5800 clinical chemistry analyzer and eGFR_{cystatin} was calculated from cystatin measured by immunoturbidimetric analysis on a Siemens Advia 1800 clinical chemistry analyzer; both creatinine and cystatin were measured at the baseline
25 visit of the study. In UKB, urine albumin:creatinine ratio (UACR) was derived from creatinine measured by enzymatic analysis and albumin measured by immunoturbidimetric analysis on a Beckman Coulter AU5400 clinical chemistry analyzer on spot urine measured at the baseline visit of the study. Lipid levels were measured during a visit to the assessment center for UK Biobank participants on a Beckman Coulter analyzer or were extracted from electronic health
30 record datasets for participants from GHS. Direct LDL measurements were available for UK Biobank participants. LDL levels were estimated using the Friedewald equation for the other participating individuals. Individuals known to be on lipid lowering medication had their pre-

treatment LDL estimated using a correction factor for individuals known to be on lipid lowering medication. Prior to genetic association analysis, continuous phenotype values were transformed by the inverse standard normal function, applied within each ancestry group and separately in men and women. Proteomic profiling was conducted on blood plasma samples collected at baseline using the Olink® Explore 1536 platform, measuring 1,472 protein analytes, capturing 1,463 unique proteins. Circulating levels of proteins were expressed as Olink's relative quantification unit (i.e., Normalized Protein eXpression) on a log-2 scale ("doi.org/10.1101/2022.06.17.496443").

Kidney disease outcomes were defined according to the International Classification of Diseases Tenth Revision (ICD-10) and read codes stored in EHRs. Self-reported disease status was used when available. Office of Population Censuses and Surveys Classification of Interventions and Procedures version 4 (OPCS4) codes were used for medical procedures in UKB.

Individuals with kidney diseases were identified as described in Table 5, combining EHR records, self-reports and eGFR and UACR measurements. Associations of circulating ANGPTL3 were assessed with incident occurrence of physician-diagnosed CKD in UKB, defined as occurrence of any of the following criteria:

1. ICD10: N18,Y841,Z49,Z992,T861,Z940
2. ICD9: 585
3. Self-reported illness code: 1580,1581,1582

For this analysis, individuals were excluded from the study population if they met any of the following criteria: prior history of physician-diagnosed CKD according to the above definition, abnormal $\text{eGFR}_{\text{creatinine}}$ defined here as $< 90 \text{ ml/min/1.72m}^2$ or $> 110 \text{ ml/min/1.72m}^2$.

For genetic analyses, participants were excluded from the control population for all kidney disease outcomes if they met any of the following criteria:

1. ICD10: N00,N01,N02,N03,N04,N05,N06,N07,N08,N10,N11,N12,N13,N14,N15, N16,N17,N18,N19,N20,N21,N22,N23,N25,N26,N27,N28,N29,Q60,Q61,Q63,C64,R944,I12, I13,T861,Z940,O904,O084,T824,Y841,Z490,Z491,Z492,Z992
2. $\text{eGFR} < 90 \text{ ml/min/1.72m}^2$
3. Self-reported illness code (UKB-specific): 1192,1193,1194,1197,1519,1520,1607, 1608,1609,1427,1405
4. Self-reported operation code (UKB-specific): 1195,1197,1476,1487,1580,1581, 1582,1618

5. OPCS4 operation code (UKB-specific): M01,M02,M03,M04,M05,M061,M068,M069,M07,M08,M09,M10,M111,M112,M131,M132,M133,M137,M14,M164,L746,M172,M174,M178,M179,X401,X402,X403,X404,X405,X406,X408,X409,X411,X412,X418,X419,X421,X428,X429,M012,M013,M014,M015,M018,M019,M084

- 5 6. Source of end-stage renal disease report code (UKB-specific): 0,1,2
7. Urinary albumin:creatinine ratio > 10 mg/g (UKB-specific)
8. EHR code for kidney transplant or dialysis in billing procedures or surgery (GHS-specific).

Table 10: Definitions of kidney disease and outcomes in UKB and GHS cohort

Kidney disease outcome	Case definition Participants were included as a case in genetic analyses if they met any of the criteria specific to each disease
Microalbuminuria	UKB.UACR > 30 mg/g
Any kidney disease	ICD10: N00,N01,N02,N03,N04,N05,N06,N07,N08,N10,N11,N12,N13,N14,N15,N16,N17,N18,N19,N25,N26,N27,N28,N29,Q60,Q61,Q63,C64,R944,I12,I13,T861,Z940,O904,O084,T824,Y841,Z490,Z491,Z492,Z992 UKB.f.20002: 1192,1193,1194,1519,1520,1607,1608,1609,1427,1405 UKB.f.20004: 1195,1476,1487,1580,1581,1582,1618 UKB.OPCS4: M01,M02,M03,M04,M05,M068,M069,M078,M079,M08,M10,M111,M112,M131,M132,M133,M137,M164,L746,M172,M174,M178,M179,X401,X402,X403,X404,X405,X406,X408,X409,X411,X412,X418,X419,X421,X428,X429,M012,M013,M014,M015,M018,M019,M084 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant or dialysis in billing procedures or surgery

	eGFR < 60 ml/min/1.72m² UKB.UACR > 30 mg/g
Chronic kidney disease, any	ICD10: N18,Q611,Q612,Q613,Q615,I12,I13,T861,Z940,Z492 UKB.f.20002: 1193,1607,1427 UKB.f.20004: 1195,1476,1580,1581,1582 UKB.OPSC4: L746,M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 60 ml/min/1.72m² UKB.UACR > 300 mg/g
Chronic kidney disease, any including microalbuminuria	ICD10: N18,Q611,Q612,Q613,Q615,I12,I13,T861,Z940,Z492 UKB.f.20002: 1193,1607,1427 UKB.f.20004: 1195,1476,1580,1581,1582 UKB.OPSC4: L746,M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412

	UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 60 ml/min/1.72m² UKB.UACR > 30 mg/g
CKD stage 3+	ICD10: N183,N184,N185,N180,N186,T861,Z492,Z940,N165 UKB.f.20002: 1193 UKB.f.20004: 1476,1195,1580,1581,1582 UKB.OPSC4: M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 60 ml/min/1.72m²
CKD stage 5+	ICD10: N185,N180,N186,T861,Z492,Z940,N165 UKB.f.20002: 1193 UKB.f.20004: 1476,1195,1580,1581,1582 UKB.OPSC4: M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412

	UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant in billing procedures or surgery eGFR < 15 ml/min/1.72m²
Renal replacement therapy	ICD10: T824,T861,Y841,Z490,Z491,Z492,Z940,Z992 UKB.f.20002: 1193 UKB.f.20004: 1195,1476,1580,1581,1582 UKB.OPSC4: L746,M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X401,X402,X403,X404,X405,X406,X408,X409,X411,X412,X418,X419,X421,X428,X429 UKB.f.42027: 0,1,2 GHS.EHR: kidney transplant or dialysis in billing procedures or surgery
End stage renal disease	ICD10: N180,N186,T861,Z492,Z940,N165 UKB.f.20002: 1193 UKB.f.20004: 1476,1195,1580,1581,1582 UKB.OPSC4: M012,M013,M014,M015,M018,M019,M084,M174,M178,M179,X402,X405,X406,X411,X412 UKB.f.42027: 0,1,2

	GHS.EHR: kidney transplant in billing procedures or surgery
--	---

ICD10 indicates the 10th revision of the International Statistical Classification of Diseases and Related Health Problems; UKB.OPCS4 indicates Office of Population Censuses and Surveys (OPCS) Classification of Interventions and Procedures version 4 as used in the UK Biobank (UKB); UKB.f.20002 indicates self-reported non-cancer illness codes as used in UKB; UKB.f.20004 indicates self-reported medical procedures as used in UKB; UKB.f.42027 indicates source of end-stage renal disease code as defined by central working group for UKB; GHS.EHR indicates billing procedures or surgeries in Geisinger Health System (GHS) relevant to disease definition; UKB.UACR indicates urine albumin:creatinine ratio measured in UKB.

10 *Genotype data*

High coverage whole exome sequencing was performed as previously described (Science, 2016, 354:aaf6814; and Nature, 2020, 586, 749-756) and as summarized below. NimbleGen probes (VCRome; for part of the GHS cohort) or a modified version of the xGen design available from Integrated DNA Technologies (IDT; for the rest of GHS and other cohorts) were used for target sequence capture of the exome. A unique 6 base pair (bp) barcode (VCRome) or 10 bp barcode (IDT) was added to each DNA fragment during library preparation to facilitate multiplexed exome capture and sequencing. Equal amounts of sample were pooled prior to exome capture. Sequencing was performed using 75 bp paired-end reads on Illumina v4 HiSeq 2500 (for part of the GHS cohort) or NovaSeq (for the rest of GHS and other cohorts) instruments. Sequencing had a coverage depth (i.e., number of sequence-reads covering each nucleotide in the target areas of the genome) sufficient to provide greater than 20x coverage over 85% of targeted bases in 96% of VCRome samples and 20x coverage over 90% of targeted bases in 99% of IDT samples. Data processing steps included sample de-multiplexing using Illumina software, alignment to the GRCh38 Human Genome reference sequence including generation of binary alignment and mapping files (BAM), processing of BAM files (e.g., marking of duplicate reads and other read mapping evaluations). Variant calling was performed using the GLNexus system (DOI: 10.1101/343970). Variant mapping and annotation were based on the GRCh38 Human Genome reference sequence and Ensembl v85 gene definitions using the snpEff software. The snpEff predictions that involve protein-coding transcripts with an annotated start and stop were then combined into a single functional impact prediction by

selecting the most deleterious functional effect class for each gene. The hierarchy (from most to least deleterious) for these annotations was frameshift, stop-gain, stop-loss, splice acceptor, splice donor, stop-lost, in-frame indel, missense, other annotations. Predicted LOF genetic variants included: a) insertions or deletions resulting in a frameshift, b) insertions, deletions or single nucleotide variants resulting in the introduction of a premature stop codon or in the loss of the transcription start site or stop site, and c) variants in donor or acceptor splice sites.

Missense variants were classified for likely functional impact according to the number of *in silico* prediction algorithms that predicted deleteriousness using SIFT (Adzhubei et al., Nat. Methods, 2010, 7, 248-9) and Polyphen2_HVAR (Adzhubei et al., Nat. Methods, 2010, 7, 248-9), LRT (Chun et al., Genome Res., 2009, 19, 1553-61) and MutationTaster (Schwarz et al., Nat. Methods, 2010, 7, 575-6). For each gene, the alternative allele frequency (AAF) and functional annotation of each variant determined inclusion into these 7 gene burden exposures: 1) pLOF variants with AAF < 1%; 2) pLOF or missense variants predicted deleterious by 5/5 algorithms with AAF < 1%; 3) pLOF or missense variants predicted deleterious by 5/5 algorithms with AAF < 0.1%; 4) pLOF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 1%; 5) pLOF or missense variants predicted deleterious by at least 1/5 algorithms with AAF < 0.1%; 6) pLOF or any missense with AAF < 1%; 7) pLOF or any missense variants with AAF < 0.1%.

Association analysis of single nucleotide polymorphisms or gene burden of rare pLOF and missense variations

Associations between phenotypes and either the burden of rare predicted loss-of-function or missense variants in a given gene, or single nucleotide polymorphism low-frequency coding variants, were examined by fitting a linear (for quantitative traits) or firth bias-corrected logistic (for binary traits) regression model adjusted for a polygenic score that approximates a genomic kinship matrix using REGENIE (Mbatchou et al., Nat. Genetics, 2020, 53, 1097–1103). Analyses were stratified by ancestry and adjusted for age, age², sex, age-by-sex and age²-by-sex interaction terms, experimental batch-related covariates, 10 common variant-derived principal components, and 20 rare variant-derived principal components. Results across cohorts for each variant-phenotype association were combined using fixed effects inverse variance weighted meta-analysis. In gene burden tests, all individuals are labeled as heterozygotes if they carry one or more qualifying rare variant (as described above based on frequency and functional annotation) and as homozygotes if they carry any qualifying variant in the homozygous state.

This “composite genotype” is then used to test for association. For analyses comparing gene burden effects on LDL or triglycerides to that on eGFR, the gene burden containing missense variants with the strongest effect (largest effect size and most significant p-value) on the relevant lipid for each gene listed was evaluated for its effect on eGFR; the burden of pLOF variants with AAF < 1% for each gene was also evaluated.

Association analysis of circulating levels of ANGPTL3 and risk of newly-onset chronic kidney disease

Participants were included if they: 1) had no prior history of physician-diagnosed CKD, or abnormal baseline eGFR_{creatinine} (defined here as < 90 ml/min/1.72m² or > 110 ml/min/1.72m²; 2) had complete information on covariates, including baseline measurements of systolic blood pressure, HDL cholesterol, LDL cholesterol (directly-measured)), history of diabetes, alcohol consumption, and smoking status; 3) had Olink assay-measured baseline levels of ANGPTL3.

Overall, 38,043 participants were included in the analysis, including 977 newly-onset CKD outcomes. Participants were grouped into quartiles based on their baseline levels of ANGPTL3. Compared to the reference group (defined here as the top quartile), hazard ratios and corresponding 95% confidence interval (Cis) were estimated using Cox proportional regression model with duration (time-on-study) as the underlying timescale, stratified by sex and study center, adjusted for age-at-baseline, systolic blood pressure (continuous), HDL cholesterol (continuous), LDL cholesterol (continuous), history of diabetes (Yes vs. No), alcohol consumption (Current-, Ex- and Never-drinkers), and smoking status (Current-, Ex- and Never-smokers). Sensitivity analyses were performed by further adjusting for baseline triglycerides and hemoglobin A1C in addition to the previous adjustments.

Example 5: Circulating ANGPTL3 is Increased in Rodent Models of Kidney Disease

Uninephrectomy of *db/db* mice, which have defective leptin receptor, on the C57BLKS/J stain background, is a common model of the diabetic nephropathy presenting with albuminuria and reduced estimated glomerular filtration rate (Tesch et al., Am. J. Physiol. Renal. Physiol., 2011, 300, F301-F310). Male *db/db* C57BLKS/J mice were uninephrectomized at 8 weeks of age under ketamine/xylazine anesthesia. Urine was collected for 16–18 hours using diuresis cages (Tecniplast) at 22 weeks of age. Urinary albumin was measured using a commercially available kit (Albuwell M, Ethos Bioscience). The urinary albumin levels were

normalized with urinary creatinine levels (Ethos Bioscience). Serum ANGPTL3 levels collected at 22 weeks of age were measured using a commercially available ELISA (BioTechne).

At 22 weeks old, the uninephrectomized *db/db* mice exhibited signs of kidney disease, including significantly increased urine albumin, compared to wildtype lean controls ($p=0.0058$, Figure 2, Panel A). At this timepoint, serum ANGPTL3 was also significantly increased 3.35-fold compared to wildtype controls ($p=0.0011$, Figure 2, Panel B).

The remnant kidney model (RKM) or 5/6ths nephrectomy is a common model of chronic kidney disease (Leelahavanichkul et al., *Kidney Int.*, 2010, 78(11), 1136-1153). 2/3 of left kidney was resected in 129sv mice (Taconic), followed by uninephrectomy of the right kidney one week later under ketamine/xylazine anesthesia. Urine was collected for 16–18 hours using diuresis cages (Tecniplast) 5 weeks after the second surgery. Urinary albumin was measured using a commercially available kit (Albuwell M, Ethos Bioscience). The urinary albumin levels were normalized with urinary creatinine levels (Ethos Bioscience). Serum ANGPTL3 levels collected 5 weeks after the second surgery were measured using a commercially available ELISA (BioTechne).

The urinary albumin levels were significantly increased in the RKM mice at 5 weeks post-second surgery compared to sham-operated controls ($p=0.0122$, Figure 3, Panel A) indicating kidney injury. At this timepoint, serum ANGPTL3 was also significantly increased ~1.4-fold in mice with the remnant kidney model compared to sham-operated controls ($p<0.0005$, Figure 3, Panel B).

In summary, circulating ANGPTL3 levels are increased in multiple models of chronic kidney disease in rodents.

Various modifications of the described subject matter, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, and the like) cited in the present application is incorporated herein by reference in its entirety and for all purposes.

What is Claimed is:

1. A method of identifying a subject having an increased risk of developing a kidney disease, the method comprising:

determining or having determined the presence or absence of an ANGPTL3 predicted

5 loss-of-function or missense variant nucleic acid molecule in a biological sample obtained from the subject, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region;

wherein:

when the subject is ANGPTL3 reference, then the subject has an

10 increased risk of developing a kidney disease; and

when the subject is heterozygous or homozygous for the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, then the subject has a decreased risk of developing a kidney disease, wherein the kidney disease is not nephrotic syndrome.

15 2. The method according to claim 1, wherein the kidney disease is chronic kidney disease, a kidney stone, chronic glomerulonephritis, nephronophthisis, chronic interstitial nephritis, or nephrosclerosis.

3. The method according to claim 1 or claim 2, wherein the ANGPTL3 predicted loss-of-function variant nucleic acid molecule is a splice-site variant, a stop-gain variant, a start-loss variant, a stop-loss variant, a frameshift variant, an in-frame indel variant, or a variant that encodes a truncated ANGPTL3 predicted loss-of-function polypeptide.

4. Use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises an inhibitory nucleic acid molecule that hybridizes to an ANGPTL3 nucleic acid molecule.

5. The use according to claim 4, wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule, a small interfering RNA (siRNA), or a short hairpin RNA (shRNA).

6. The use according to claim 5, wherein the inhibitory nucleic acid molecule comprises an antisense nucleic acid molecule.

7. The use according to claim 5, wherein the inhibitory nucleic acid molecule comprises a small interfering RNA (siRNA).

5 8. The use according to claim 5, wherein the inhibitory nucleic acid molecule comprises a short hairpin RNA (shRNA).

9. Use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid
10 molecule, wherein the kidney disease is not nephrotic syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises a Cas protein and guide RNA (gRNA) that hybridizes to a gRNA recognition sequence within an ANGPTL3 genomic nucleic acid molecule.

15 10. Use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule, wherein the kidney disease is not nephrotic syndrome, wherein the ANGPTL3 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in
20 the coding region, and wherein the ANGPTL3 inhibitor comprises a small molecule.

11. Use of an Angiopoietin Like 3 (ANGPTL3) inhibitor in the manufacture of a medicament for the treatment and/or prevention of a kidney disease in a subject that is ANGPTL3 reference, or is heterozygous for ANGPTL3 predicted loss-of-function or missense variant nucleic acid
25 predicted loss-of-function or missense variant nucleic acid molecule comprises a variation in the coding region, and wherein the ANGPTL3 inhibitor comprises an antibody.