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(54) **Title:** USE OF INHIBITORS OF CUBILIN FOR THE TREATMENT OF CHRONIC KIDNEY DISEASES

(57) **Abstract:** Chronic Kidney Disease (CKD) is a major burden of public health affecting millions of people around the world. Even though many aspects of the complex mechanisms orchestrating progression of renal disease have been identified, so far there is no specific treatment to slow down or prevent CKD progression. *CUBN* encodes for cubilin that is a proximal tubular uptake receptor with 27 CUB domains for ligand binding. The inventors now identify 26 patients, in whom rare biallelic *CUBN* variants are associated with isolated proteinuria. Yet, renal function was normal in all cases. Most of the 35 novel *CUBN* variants were localized to C-terminal CUB domains of the protein, unlike the more N-terminal I-GS mutations. Finally, the inventors show that four low-frequency and common C-terminal *CUBN* variants with strong albuminuria associations in GWAS studies are associated with slightly higher eGFR in a meta-analysis of over half a million individuals from the general population compared to their ancestral alleles. Collectively, the data suggest that *CUBN*-associated albuminuria is an unexpectedly common benign condition that does not require any treatment and may even have protective effects with respect to renal filtration function. More importantly, the results suggest that inhibitor of cubilin would be suitable for the treatment of CKD, particularly those CKD forms in which glomerular proteinuria is causing tubular damage due to albumin overload.

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USE OF INHIBITORS OF CUBILIN FOR THE TREATMENT OF CHRONIC KIDNEY DISEASES

FIELD OF THE INVENTION:

The present invention is in the field of medicine, in particular nephrology.

BACKGROUND OF THE INVENTION:

Chronic Kidney Disease (CKD) is a major burden of public health affecting millions of people around the world. It is rising in prevalence with high public health costs and is associated with a high degree of morbidity and mortality. Diabetes and hypertension are the predominant causes of CKD, accounting for approximately 44% and 30% of incident CKD cases. Even though many aspects of the complex mechanisms orchestrating progression of renal disease have been identified, so far there is no specific treatment to slow down or prevent CKD progression.

An important hallmark of many CKD forms is the loss of proteins into the urine or proteinuria. The main cause of proteinuria is the dysfunction of the glomerular filtration barrier. Being an important risk factor for renal and cardiovascular disease [1], proteinuria not only affects the composition of the proteins in the serum but also leads to an overload of the renal tubules. Particularly damaging is the uptake of albumin because albumin can carry fatty acids that are toxic for the tubular cell organelles, such as endoplasmic reticulum (ER) and mitochondria. Anti-proteinuric therapy, for example through angiotensin-converting-enzyme (ACE) inhibition, is therefore an important renoprotective therapy [2].

Another form of proteinuria is caused by defects in the proximal tubular protein reabsorption. Tubular proteinuria is of smaller range than glomerular proteinuria because it only affects the proteins that are filtered by the glomerulus. Typically, these are proteins, such as beta2-microglobulin, with a smaller size than albumin [3]. Albumin itself is filtered to a limited extent and usually accounts for less than half of the urinary protein in tubular proteinuria. The uptake of the filtered proteins is carried out by a receptor complex on the apical membrane of proximal tubular cells, consisting of megalin (gene name: *LRP2*), cubilin (*CUBN*) and amnionless (*AMN*) [4]. In the human kidney, all three proteins are exclusively expressed in proximal tubules [5]. Megalin and amnionless are type I transmembrane proteins, whereas cubilin is a peripheral protein that requires amnionless for anchoring to the membrane.

Anchoring occurs via beta-helix-beta-helix association between amnionless and the N-terminal hydrophobic stretches of three cubilin subunits [6]. Each cubilin protomer has 8 EGF domains and 27 CUB domains, some of which are involved in Ca^{2+}-dependent ligand binding [6, 7].

While mutations in *LRP2* cause Donnai-Barrow syndrome, a multi-system developmental disorder, *CUBN* and *AMN* mutations lead to I-GS, featured by intestinal vitamin B12-malabsorption disorder and in about half of the cases proteinuria [8]. Most I-GS mutations are in the N-terminal half, either affecting the interaction with AMN or the CUB domains 5-8 (CUB5-8; CUB stands for *C*omplement C1r/C1s, *U*egf (epidermal growth factor-related sea urchin protein) and *B*one morphogenic protein-1) that bind vitamin B12/intrinsic factor (IF) [6]. Interestingly, one individual with a homozygous deletion of exon 53 harbouring CUB20 was shown to have isolated proteinuria [9]. This is consistent with recent genome-wide association studies (GWAS) showing associations of C-terminal *CUBN* variants and albuminuria [10-12]. These studies suggest that the C-terminal half of cubilin is important for preventing urinary albumin loss in humans. However, it is unclear whether the resulting albuminuria impairs renal function.

SUMMARY OF THE INVENTION:

As defined by the claims, the present invention relates to use inhibitors of cubilin for the treatment of chronic kidney diseases.

DETAILED DESCRIPTION OF THE INVENTION:

The first object of the present invention relates to a method of treating a chronic kidney disease in a patient in need thereof comprising administering to the patient a therapeutically effective amount of an inhibitor of cubilin.

As used herein, the term “chronic kidney disease” or “CKD” has its general meaning in the art and refers to a progressive loss in renal function over a period of months or years. CKD is used to classify numerous conditions that affect the kidney, destruction of the renal parenchyma and the loss of functional nephrons or glomeruli. It should be further noted that CKD can result from different causes, but the final pathway remains renal fibrosis. CKD is defined as kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for 3 months or more, irrespective of cause. GFR can be estimated from calibrated serum creatinine and estimating equations, such as the Modification of Diet in Renal Disease (MDRD) Study equation or the Cockcroft-Gault formula. Kidney disease severity is classified into five stages

according to the level of GFR. Examples of etiology of CKD include, but are not limited to, cardiovascular diseases, hypertension, diabetes, glomerulonephritis, polycystic kidney diseases, and kidney graft rejection.

As used herein, the term "*glomerular filtration rate*" or "GFR" refers to the volume of fluid filtered from the renal (kidney) glomerular capillaries into the Bowman's capsule per unit time. GFR is used to assess renal function in a subject. As used herein, the term "estimated GFR" or "eGFR" refers to an estimate of the Glomerular Filtration Rate or GFR, calculated using the Modification of Diet in Renal Disease (MDRD) equation developed by the Modification of Diet in Renal Disease Study Group described in Levey A S, Bosch J P, Lewis J B, Greene T, Rogers N, Roth D, "A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group" Ann. Intern. Med. 130 (6): 461-70 (1999), the contents of which are herein incorporation by reference. Typically, the unit of measurement for eGFR is mL/min/1,73m^2. Typically, the eGFR typically comprises between 0 and 120 mL/min/1,73m^2.

The inhibitors of the present invention are particularly suitable for increasing the renal function in patients with glomerular proteinuria.

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of patient at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a patient having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a patient beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a patient during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a

greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a patient during treatment of an illness, e.g., to keep the patient in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., pain, disease manifestation, etc.]).

As used herein, the term “cubilin” has its general meaning in the art and refers to the protein encoded by the CUBN gene. An exemplary amino acid sequence for cubilin is as set forth in SEQ ID NO:1.

SEQ ID NO:1 >sp|O60494|CUBN_HUMAN Cubilin OS=Homo sapiens OX=9606 GN=CUBN PE=1 SV=5

MMNMSLPFLWSLLTLLIFAEVNGEAGELELQRQKRSINLQQPRMATERGNLVFLTGSAQN
IEFRTGSLGKIKLNDEDLSECLHQIQKNKEDIIELKGSAIGLPQNISSQIYQLNSKLVDL
ERKFQGLQQTVDKKVCSSNPCQNGGTCLNLHDSFFCICPPQWKGPLCSADVNECEIYSGT
PLSCQNGGTCVNTMGSYSCHCPPETYGPQCASKYDDCEGGSVARCVHGICEDLMREQAGE
PKYSCVCDAGWMFSPNSPACTLDRDECSFQPGPCSTLVQCFNTQGSFYCGACPTGWQGNG
YICEDINECEINNGGCSVAPPVECVNTPGSSHCQACPPGYQGDGRVCTLTDICSVSNGGC
HPDASCSSTLGSLPLCTCLPGYTGNGYGPNGCVQLSNICLSHPCLNGQCIDTVSGYFCKC
DSGWTGVNCTENINECLSNPCLNGGTCVDGVDSFSCECTRLWTGALCQVPQQVCGESLSG
INGSFSYRSPDVGYVHDVNCFWVIKTEMGKVLRITFTFFRLESMDNCPHEFLQVYDGDSS
SAFQLGRFCGSSLPHELLSSDNALYFHLYSEHLRNGRGFTVRWETQQPECGGILTGPYGS
IKSPGYPGNYPPGRDCVWIVVTSPDLLVTFTFGTLSLEHHDDCNKDYLEIRDGPLYQDPL
LGKFCTTFSVPPLQTTGPFARIHFHSDSQISDQGFHITYLTSPSDLRCGGNYTDPEGELF
LPELSGPFTHTRQCVYMMKQPQGEQIQINFTHVELQCQSDSSQNYIEVRDGETLLGKVCG
NGTISHIKSITNSVWIRFKIDASVEKASFRAVYQVACGDELTGEGVIRSPFFPNVYPGER
TCRWTIHQPQSQVILLNFTVFEIGSSAHCETDYVEIGSSSILGSPENKKYCGTDIPSFIT
SVYNFLYVTFVKSSSTENHGFMAKFSAEDLACGEILTESTGTIQSPGHPNVYPHGINCTW
HILVQPNHLIHLMFETFHLEFHYNCTNDYLEVYDTDSETSLGRYCGKSIPPSLTSSGNSL
MLVFVTDSDLAYEGFLINYEAISAATACLQDYTDDLGTFTSPNFPNNYPNNWECIYRITV
RTGQLIAVHFTNFSLEEAIGNYYTDFLEIRDGGYEKSPLLGIFYGSNLPPTIISHSNKLW
LKFKSDQIDTRSGFSAYWDGSSTGCGGNLTTSSGTFISPNYPMPYYHSSECYWWLKSSHG
SAFELEFKDFHLEHHPNCTLDYLAVYDGPSSNSHLLTQLCGDEKPPLIRSSGDSMFIKLR
TDEGQQGRGFKAEYRQTCENVVIVNQTYGILESIGYPNPYSENQHCNWTIRATTGNTVNY
TFLAFDLEHHINCSTDYLELYDGPRQMGRYCGVDLPPPGSTTSSKLQVLLLTDGVGRREK
GFQMQWFVYGCGGELSGATGSFSSPGFPNRYPPNKECIWYIRTDPGSSIQLTIHDFDVEY
HSRCNFDVLEIYGGPDFHSPRIAQLCTQRSPENPMQVSSTGNELAIRFKTDLSINGRGFN
ASWQAVTGGCGGIFQAPSGEIHSPNYPSPYRSNTDCSWVIRVDRNHRVLLNFTDFDLEPQ
DSCIMAYDGLSSTMSRLARTCGREQLANPIVSSGNSLFLRFQSGPSRQNRGFRAQFRQAC
GGHILTSSFDTVSSPRFPANYPNNQNCSWIIQAQPPLNHITLSFTHFELERSTTCARDFV
EILDGGHEDAPLRGRYCGTDMPHPITSFSSALTLRFVSDSSISAGGFHTTVTASVSACGG
TFYMAEGIFNSPGYPDIYPPNVECVWNIVSSPGNRLQLSFISFQLEDSQDCSRDFVEIRE
GNATGHLVGRYCGNSFPLNYSSIVGHTLWVRFISDGSGSGTGFQATFMKIFGNDNIVGTH
GKVASPFWPENYPHNSNYQWTVNVNASHVVHGRILEMDIEEIQNCYYDKLRIYDGPSIHA

RLIGAYCGTQTESFSSTGNSLTFHFYSDSSISGKGFLLEWFAVDAPDGVLPTIAPGACGG
FLRTGDAPVFLFSPGWPDSYSNRVDCTWLIQAPDSTVELNILSLDIESHRTCAYDSLVIR
DGDNNLAQQLAVLCGREIPGPIRSTGEYMFIRFTSDSSVTRAGFNASFHKSCGGYLHADR
GIITSPKYPETYPSNLNCSWHVLVQSGLTIAVHFEQPFQIPNGDSSCNQGDYLVLRNGPD
ICSPPLGPPGGNGHFCGSHASSTLFTSDNQMFVQFISDHSNEGQGFKIKYEAKSLACGGN
VYIHDADSAGYVTSPNHPHNYPPHADCIWILAAPPETRIQLQFEDRFDIEVTPNCTSNYL
ELRDGVDSDAPILSKFCGTSLPSSQWSSGEVMYLRFRSDNSPTHVGFKAKYSIAQCGGRV
PGQSGVVESIGHPTLPYRDNLFCEWHLQGLSGHYLTISFEDFNLQNSSGCEKDFVEIWDN
HTSGNILGRYCGNTIPDSIDTSSNTAVVRFVTDGSVTASGFRLRFESSMEECGGDLQGSI
GTFTSPNYPNPNPHGRICEWRITAPEGRRITLMFNNLRLATHPSCNNEHVIVFNGIRSNS
PQLEKLCSSVNVSNEIKSSGNTMKVIFFTDGSRPYGGFTASYTSSEDAVCGGSLPNTPEG
NFTSPGYDGVRNYSRNLNCEWTLSNPNQGNSSISIHFEDFYLESHQDCQFDVLEFRVGDA
DGPLMWRLCGPSKPTLPLVIPYSQVWIHFVTNERVEHIGFHAKYSFTDCGGIQIGDSGVI
TSPNYPNAYDSLTHCSSLLEAPQGHTITLTFSDFDIEPHTTCAWDSVTVRNGGSPESPII
GQYCGNSNPRTIQSGSNQLVVTFNSDHSLQGGGFYATWNTQTLGCGGIFHSDNGTIRSPH
WPQNFPENSRCSWTAITHKSKHLEISFDNNFLIPSGDGQCQNSFVKVWAGTEEVDKALLA
TGCGNVAPGPVITPSNTFTAVFQSQEAPAQGFSASFVSRCGSNFTGPSGYIISPNYPKQY
DNNMNCTYVIEANPLSVVLLTFVSFHLEARSAVTGSCVNDGVHIIRGYSVMSTPFATVCG
DEMPAPLTIAGPVLLNFYSNEQITDFGFKFSYRIISCGGVFNFSSGIITSPAYSYADYPN
DMHCLYTITVSDDKVIELKFSDFDVVPSTSCSHDYLAIYDGANTSDPLLGKFCGSKRPPN
VKSSNNSMLLVFKTDSFQTAKGWKMSFRQTLGPQQGCGGYLTGSNNTFASPDSDSNGMYD
KNLNCVWIIIAPVNKVIHLTFNTFALEAASTRQRCLYDYVKLYDGDSENANLAGTFCGST
VPAPFISSGNFLTVQFISDLTLEREGFNATYTIMDMPCGGTYNATWTPQNISSPNSSDPD
VPFSICTWVIDSPPHQQVKITVWALQLTSQDCTQNYLQLQDSPQGHGNSRFQFCGRNASA
VPVFYSSMSTAMVIFKSGVVNRNSRMSFTYQIADCNRDYHKAFGNLRSPGWPDNYDNDKD
CTVTLTAPQNHTISLFFHSLGIENSVECRNDFLEVRNGSNSNSPLLGKYCGTLLPNPVFS
QNNELYLRFKSDSVTSDRGYEIIWTSSPSGCGGTLYGDRGSFTSPGYPGTYPNNTYCEWV
LVAPAGRLVTINFYFISIDDPGDCVQNYLTLYDGPNASSPSSGPYCGGDTSIAPFVASSN
QVFIKFHADYARRPSAFRLTWDS

As used herein, the term “inhibitor of cubilin” refers to a molecule that partially or fully blocks, inhibits, or neutralizes a biological activity or expression of cubilin. An inhibitor of cubilin can be a molecule of any type that interferes with the ability of cubilin for binding to its ligands (e.g. albumin), for example, either by decreasing transcription or translation of cubilin encoding nucleic acid, or by inhibiting or blocking cubilin activity, or both. In some examples, an inhibitor of cubilin is an agent that interferes with the capability of cubilin to bind to its ligands such as albumin. Examples of inhibitor of cubilins include, but are not limited to, antisense polynucleotides, interfering RNAs, catalytic RNAs, RNA-DNA chimeras, cubilin - specific aptamers, anti-cubilin antibodies, cubilin-binding fragments of anti-cubilin antibodies, cubilin-binding small molecules, cubilin-binding peptides, and other polypeptides that specifically bind cubilin (including, but not limited to, cubilin-binding fragments of one or more cubilin ligands, optionally fused to one or more additional domains), such that the interaction between the inhibitor of cubilin and cubilin results in a reduction or cessation of cubilin activity or expression.

In particular, the inhibitor of cubilin is a small molecule, such as a small organic molecule, which typically has a molecular weight less than 5,000 kDa.

In some embodiments, the inhibitor of cubilin is an antibody.

As used herein the term "antibody" or "immunoglobulin" have the same meaning, and will be used equally in the present invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments.

As used herein, the term “specificity” refers to the ability of an antibody to detectably bind an epitope presented on an antigen, such as a cubilin, while having relatively little detectable reactivity with non-cubilin proteins or structures (such as other proteins presented on T cells, or on other cell types). Specificity can be relatively determined by binding or competitive binding assays, using, e.g., Biacore instruments, as described elsewhere herein. Specificity can be exhibited by, e.g., an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules (in this case the specific antigen is a cubilin polypeptide). The term “affinity”, as used herein, means the strength of the binding of an antibody to an epitope. The affinity of an antibody is given by the dissociation constant Kd, defined as [Ab] x [Ag] / [Ab-Ag], where [Ab-Ag] is the molar concentration of the antibody-antigen complex, [Ab] is the molar concentration of the unbound antibody and [Ag] is the molar concentration of the unbound antigen. The affinity constant Ka is defined by 1/Kd. Preferred methods for determining the affinity of mAbs can be found in Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol. 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of mAbs is the use of Biacore instruments.

In some embodiments, the antibody of the present invention has specificity for the C-terminal CUB domains of cubilin, because based on the identified patient mutations the albumin binding site should be in the C-terminal half.

The term “binding” as used herein refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. In particular, as used herein, the term "binding" in the context of the binding of an antibody to a predetermined target molecule (e.g. an antigen or epitope) typically is a binding with an affinity corresponding to a K_D of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less.

In some embodiments, the antibody of the present invention is a chimeric antibody. As used herein, the term "chimeric antibody" refers to an antibody that comprises a VH domain and a VL domain of a non-human antibody, and a CH domain and a CL domain of a human antibody.

In some embodiments, the antibody of the present invention is a humanized antibody. The term "humanized antibody" refers to an antibody having variable region framework and constant regions from a human antibody but retains the CDRs of a previous non-human antibody. In some embodiments, a humanized antibody contains minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof may be human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity.

In some embodiments, the antibody of the present invention is a human antibody. As used herein the term "human monoclonal antibody", is intended to include antibodies having variable and constant regions derived from human immunoglobulin sequences. The human antibodies of the present invention may include amino acid residues not encoded by human immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). More specifically, the term "human monoclonal antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

In some embodiments, the antibody of the present invention is a single domain antibody. As used herein the term “single domain antibody” has its general meaning in the art and refers to the single heavy chain variable domain of antibodies of the type that can be found in Camelid mammals which are naturally devoid of light chains. Such single domain antibody are also “nanobody®”. For a general description of (single) domain antibodies, reference is also made to the prior art cited above, as well as to EP 0 368 684, Ward et al. (Nature 1989 Oct 12; 341 (6242): 544-6), Holt et al., Trends Biotechnol., 2003, 21(11):484-490; and WO 06/030220, WO 06/003388. The amino acid sequence and structure of a single domain antibody can be considered to be comprised of four framework regions or "FRs" which are referred to in the art and herein as "Framework region 1" or "FRl "; as "Framework region 2" or "FR2"; as "Framework region 3 " or "FR3"; and as "Framework region 4" or “FR4” respectively; which framework regions are interrupted by three complementary determining regions or "CDRs", which are referred to in the art as "Complementarity Determining Region for "CDRl”; as "Complementarity Determining Region 2" or "CDR2” and as "Complementarity Determining Region 3" or "CDR3", respectively. Accordingly, the single domain antibody can be defined as an amino acid sequence with the general structure : FRl - CDRl - FR2 - CDR2 - FR3 - CDR3 - FR4 in which FRl to FR4 refer to framework regions 1 to 4 respectively, and in which CDRl to CDR3 refer to the complementarity determining regions 1 to 3. In the context of the invention, the amino acid residues of the single domain antibody are numbered according to the general numbering for VH domains given by the IMGT numbering system (Lefranc M.-P., "Unique database numbering system for immunogenetic analysis" Immunology Today, 18, 509 (1997)). The IMGT unique numbering has been defined to compare the variable domains whatever the antigen receptor, the chain type, or the species (Lefranc M.-P., "Unique database numbering system for immunogenetic analysis" Immunology Today, 18, 509 (1997) ; Lefranc M.-P., "The IMGT unique numbering for Immunoglobulins, T cell receptors and Ig-like domains" The Immunologist, 7, 132-136 (1999).; Lefranc, M.-P., Pommié, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V. and Lefranc, G., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains" Dev. Comp. Immunol., 27, 55-77 (2003).). In the IMGT unique numbering, the conserved amino acids always have the same position, for instance cysteine 23, tryptophan 41, hydrophobic amino acid 89, cysteine 104, phenylalanine or tryptophan 118. The IMGT unique numbering provides a standardized delimitation of the framework regions (FR1-IMGT: positions 1 to 26, FR2-IMGT: 39 to 55, FR3-IMGT: 66 to 104 and FR4-IMGT: 118 to 128) and of the complementarity determining regions: CDR1-IMGT: 27 to 38, CDR2-IMGT: 56 to 65

and CDR3-IMGT: 105 to 117. As gaps represent unoccupied positions, the CDR-IMGT lengths become crucial information. Gaps in the CDR1-IMGT and CDR2-IMGT (less than 12 and 10 amino acid long, respectively) are put at the top of the CDR-IMGT loops. For instance, when the length of CDR1-IMGT is 7 amino acids, it comprises the positions 27, 28, 29, 30, 36, 37 and 38. When the length of CDR2-IMGT is 7 amino acids, it comprises the positions 56, 57, 58, 59, 63, 64, and 65. The basic length of a rearranged CDR3-IMGT is 13 amino acids (positions 105 to 117), which corresponds to a JUNCTION of 15 amino acids (2nd-CYS 104 to J-TRP or J-PHE 118). This length and corresponding numbering were chosen since they are convenient to use. Indeed, 80% of the IG and TR rearranged sequences in IMGT/LIGM-DB have a CDR3-IMGT length less than or equal to 13 amino acids. If the CDR3-IMGT length is less than 13 amino acids, gaps are created from the top of the loop, in the following order 111, 112, 110, 113, 109, 114, etc. Accordingly, when the length of CDR3-IMGT is 9 amino acids, it comprises the positions 105; 106; 107; 108; 109; 114; 115; 116; and 117. When length of CDR3-IMGT is 9 amino acids, it comprises the positions 105; 106; 107; 108; 109; 110; 112; 113; 114; 115; 116; and 117. If the CDR3-IMGT length is more than 13 amino acids, additional positions are created between positions 111 and 112 at the top of the CDR3-IMGT loop in the following order 112.1,111.1, 112.2, 111.2, 112.3, 111.3, etc. Accordingly when the length of CDR3-IMGT is 15 amino acids, it comprises the additional positions 111.1 and 112.1.

In some embodiments, the single domain antibody of the present invention is characterized by the same frameworks regions FR1-FR4 as described in Table A.

Table A: frameworks regions FR1-FR4 (IMGT) of the single domain antibodies of the present invention:

Framework region	Sequence
FR1	VQLQASGGGFVQPGGSLRLSCAASG (SEQ ID NO:2)
FR2	MGWFRQAPGKEREFVSAISS (SEQ ID NO:3)
FR3	YYADSVKGRFTISRDNSKNTVYLQMNSLRAEDTATYYCA (SEQ ID NO:4)
FR4	YWGQGTQVTVSS (SEQ ID NO:5)

In some embodiments, the inhibitor of cubilin is an inhibitor of cubilin expression. An "inhibitor of expression" refers to a natural or synthetic compound that has a biological effect to inhibit the expression of a gene. In some embodiments, said inhibitor of gene expression is a siRNA, an antisense oligonucleotide or a ribozyme. For example, anti-sense oligonucleotides, including anti-sense RNA molecules and anti-sense DNA molecules, would act to directly block the translation of cubilin mRNA by binding thereto and thus preventing protein translation or increasing mRNA degradation, thus decreasing the level of cubilin, and thus activity, in a cell. For example, antisense oligonucleotides of at least about 15 bases and complementary to unique regions of the mRNA transcript sequence encoding cubilin can be synthesized, e.g., by conventional phosphodiester techniques. Methods for using antisense techniques for specifically inhibiting gene expression of genes whose sequence is known are well known in the art (e.g. see U.S. Pat. Nos. 6,566,135; 6,566,131; 6,365,354; 6,410,323; 6,107,091; 6,046,321; and 5,981,732). Small inhibitory RNAs (siRNAs) can also function as inhibitors of expression for use in the present invention. cubilin gene expression can be reduced by contacting a patient or cell with a small double stranded RNA (dsRNA), or a vector or construct causing the production of a small double stranded RNA, such that cubilin gene expression is specifically inhibited (i.e. RNA interference or RNAi). Antisense oligonucleotides, siRNAs, shRNAs and ribozymes of the invention may be delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the antisense oligonucleotide, siRNA, shRNA or ribozyme nucleic acid to the cells and typically cells expressing cubilin. Typically, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the antisense oligonucleotide, siRNA, shRNA or ribozyme nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art. In some embodiments, the inhibitor of expression is an endonuclease. In a particular embodiment, the endonuclease is CRISPR-cas. In some embodiment, the endonuclease is CRISPR-cas9, which is from Streptococcus pyogenes. The

CRISPR/Cas9 system has been described in US 8697359 B1 and US 2014/0068797. In some embodiment, the endonuclease is CRISPR-Cpf1, which is the more recently characterized CRISPR from Provotella and Francisella 1 (Cpf1) in Zetsche et al. ("Cpf1 is a Single RNA-guided Endonuclease of a Class 2 CRISPR-Cas System (2015); Cell; 163, 1-13).

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of drug may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of drug to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the inhibitor are outweighed by the therapeutically beneficial effects. The efficient dosages and dosage regimens for drug depend on the disease or condition to be treated and may be determined by the persons skilled in the art. A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician could start doses of drug employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable dose of a composition of the present invention will be that amount of the compound, which is the lowest dose effective to produce a therapeutic effect according to a particular dosage regimen. Such an effective dose will generally depend upon the factors described above. For example, a therapeutically effective amount for therapeutic use may be measured by its ability to stabilize the progression of disease. A therapeutically effective amount of a therapeutic compound may decrease tumour size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected. An exemplary, non-limiting range for a therapeutically effective amount of drug is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, about 3 mg/kg, about 5 mg/kg or about 8 mg/kg. An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the present invention is 0.02-100 mg/kg, such as about 0.02-30 mg/kg, such as about 0.05-10 mg/kg or 0.1-3 mg/kg, for example about 0.5-2 mg/kg. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target. Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic

response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. In some embodiments, the efficacy of the treatment is monitored during the therapy, e.g. at predefined points in time. As non-limiting examples, treatment according to the present invention may be provided as a daily dosage of the agent of the present invention in an amount of about 0.1-100 mg/kg, such as 0.2, 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

Typically, the inhibitor of cubilin of the present invention is administered to the subject in the form of a pharmaceutical composition, which comprises a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene- block polymers, polyethylene glycol and wool fat.

In some embodiments, the administration of the inhibitor of cubilin is combined with an anti-proteinuric therapy. Typically said therapy comprises angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin receptor blockers (ARBs). Suitable ACE inhibitors include, but are not limited to, ramipril (Altace®), quinapril HCl (Accupril®), captopril (Capoten®), benazapril HCl (Lotrel®, Lotensin®), trandolapril (Mavik®, Tarka®), fosinopril (Monpril®), moexipril HCl (UnivascE, Uniretic®), enalopril maleate (Vasotec®, Lexxel®, Teczem®, Vaseretic®), lisinopril (Zestrilg, Zestoretic®, Prinivil®, Prinzide®); and the like. Orally active ACE inhibitors will be used in some embodiments, where orally active ACE inhibitors include, ramipril, enalapril, captopril, alacepril, benazepril, ceranapril, cilazapril,

delapril, fosinopril, imidapril, libenzapril, lisinopril, moexipril, moveltipril, perindopril, quinapril, spirapril, zofenopril, trandolapril, BPL 36378, CS 622, FPL 63547, S 9650 and others. Orally active ACE inhibitors are described, for example, in "Pharmacology of Antihypertensive Therapeutics" (Eds. D. Ganten, P. J. Mutrow) Springer Verlag, Berlin 1990, pp. 377-480. Also suitable for use is an ACE inhibitor as described in U.S. Pat. No. 5,236,933. Suitable angiotensin receptor blockers (ARB) include, but are not limited to, candesartan (Atacand®), eprosartan (Teveten®), irbesartan (Avapro®), losartan (Cozaar®), olmesartan (Benicar®), telmisartan (Micardis®), and valsartan (Diovan).

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

EXAMPLE:

Methods:

Patients

The global cohort was approved by the Comité de Protection des Personnes Ile-De-France II and divided into two cohorts:

Genetic kidney disease cohort: 762 patients with suspected genetic renal disease sequenced by the "renome panel" (Suppl. Table 2). Diseases belonging to the proteinuric group are Alport syndrome (ALP) and steroid-resistant nephrotic syndrome (SRNS). The non-proteinuric group consists of renal tubular dysgenesis (RTD), renal hypodysplasia (HYP), tubulointerstitial nephritis (NTI), nephronophthisis (NPH) and polycystic kidney disease PK).

Chronic PU cohort: 107 selected individuals exhibiting isolated chronic proteinuria (between 0.5 and 3 g/d) but no genetic diagnosis despite the previous testing of several glomerular genes. The selected patients were heterogeneous in ages and were recruited through adult and pediatric nephrology departments in France. All the clinical information was provided and collected by clinicians prescribing the genetic testing, including familial information and pedigree.

DNA extraction and preparation for next-generation sequencing

Blood samples were collected from patients and relatives after written informed consent. Genomic DNA was extracted by standard methods and DNA quality was later evaluated as requested by the sequencer company. For this study, the 107 patients of the selected cohort were screened using our next-generation sequencing panel targeting seventeen genes chosen based on the literature (SureSelectXT, Agilent Technologies, France). For most of these genes, mutations have already been correlated with tubular proteinuric diseases, but the other genes were chosen because of being involved in key pathways of the proximal tubule function. High-throughput sequencing was carried out using a MiSeq/HiSeq platform (Illumina, San Diego, CA).

Mutation filtering

Variant annotation and analysis was performed using the Polyweb software interface provided and designed by the Bioinformatics platform of Paris Descartes University, Paris, France. All variants that were deemed functional by our filtering algorithm ("filtered variants") were verified by Sanger sequencing, and when available, parents DNA was used for segregation of heterozygous variants. In order to evaluate the pathogenic character of the identified variants, we used the guidelines of the American College of Medical Genetics [30].

For the filtering, only variants in coding regions or essential splice sites were considered in our study. Silent mutations and splice variants without consequences on the splice score were ignored. All missense mutations were predicted to be damaging with at least one out of three damage prediction algorithms Mutation Taster (http://www.mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/php2/) and SIFT (http://sift.jcvi.org/). Only for the variant localizing to the vitamin B12/IF-binding site (N1303H), no damaging effect was predicted. However, the structural simulation showed a strong effect. All variants were either absent from reference populations (gnomAD [13]) or rare with global allele frequencies between 0,00001 and 0,00177 for diagnostic and 0.00364 for discovery cohort. We considered previously reported pathogenic nonsense, frameshift, essential splice (Human Gene Mutation Database professional [14] and missense variants with high prediction scores.

Statistical analysis

Continuous values are here reported as means with ± SD (standard deviation). Dichotomous data are showed as percentages. We applied χ^2 or Fisher exact tests to dichotomous data in order to compare differences between two groups. For continuous data comparison we used an unpaired *t* test for the Gaussian sampled data. Cumulative frequencies

of variants were first calculated by combining the frequencies of all considered variants and when possible, the presence of several variants per individual was used to adjust this estimation. Two-tailed *P* values < 0.05 were regarded as statistically significant. Statistical analyses were performed using Rstudio and Prism 4 (GraphPad) software.

Structural analysis of variants

Structural models of individual cubilin CUB domains were generated from previously determined structures using the Phyre2 server [31]. Figures were prepared with PyMOL.

Lookup of genetic variants in the CKDgen Consortium

Four low-frequency or common missense variants known to be associated with albuminuria from GWAS of population-based cohorts (rs141640975, rs144360241, rs45551835, and rs1801239) [10-12, 16, 17], were evaluated for association with eGFR using summary statistics from a large-scale meta-analysis of mostly population-based studies within the CKDGen Consortium (Wuttke, Li, Li, Sieber, Feitosa, Gorski et al, Nature Genetics, 2019, *accepted*). Alleles, effect direction, standard error, p-value as well as sample size were extracted from genome-wide results. Sample size varied between 331,340 and 597,710 across variants.

Lookup of genetic variants in the meta European exome wide association study (ExWAS) with and without diabetes

Three low frequency or common missense variants (rs141640975, rs45551835 and rs1801239) associating with albuminuria from population based GWAS studies [9, 10, 15, 16] and an ExWAS (exome wide association study) [8] were evaluated for association with albuminuria and eGFR using summary statistics (alleles, effect, standard error, p value and sample size) from an ExWAS discovery meta-analysis comprising 5 studies (3 population based and 2 type 2 diabetes studies) from Denmark [8]. Albeit summary statistics for albuminuria were available for all 3 variants and for eGFR only for rs141640975, we assessed the association of other two variants (rs45551835 and rs1801239) and eGFR. Details on the methods and participating studies has been described previously [8].

Similarly, association summary for the three variants for eGFR and albuminuria was also reported, based on stratification for diabetes status. Total sample size varied between 13, 124 to 13, 550 (3837 to 3990 individuals with diabetes and 9251 to 9449 individuals without diabetes) across variants.

Results

We performed next-generation sequencing (NGS) of a panel containing 309 renal disease genes (renome panel) in a cohort of 762 patients with suspected genetic kidney disease ("genetic kidney disease cohort"; **not shown**). We grouped the patients into a non-proteinuric and a proteinuric group, with the latter consisting of suspected SRNS and Alport syndrome (**data not shown**). While synonymous and non-synonymous missense variants were equally distributed between the two groups, all protein-truncating variants (PTVs), including essential splicing, frameshift and stop-gain, were strongly enriched in the proteinuric group (**data not shown**), confirming the association of *CUBN* variants with proteinuria. Furthermore, when the number of patients carrying at least one PTV in *CUBN* was evaluated, we found that the odds of having proteinuria were 4.5 times higher when carrying at least one PTV (p=0.038, OR=4.5) (**data not shown**). Interestingly, most PTVs were after CUB8, which was consistent with the position of PTVs found in the reference genome database gnomAD [13] or in our in-house genome database.

Within this cohort, we identified thirteen patients from ten families of European or African descent with recessive mutations in the *CUBN* gene (**data not shown**). While in one half of the families homozygous mutations could be found, compound heterozygous mutations were found in the other half. Seven were protein-truncating mutations, one of which was found in two unrelated families (**data not shown**). Four variants were missense mutations with strong Polyphen-2 and SIFT scores. All variants were rare in our in-house genome database (mostly enriched for European or African ancestries) or in public reference genome databases (e.g. gnomAD [13])) with frequencies below 0.2% (**data not shown**). Unlike the I-GS mutations [14], all these novel *CUBN* variants (except for p.T55A) localized to the C-terminal half (aa: 2030-3520) (**data not shown**). To make functional predictions for the missense variants, we mapped them onto the crystal structure of cubilin [7] or *in silico* models of individual CUB domains. While all mutations were found to reside in CUB domains, a single variant (p.D3492Y) localized close to the putative Ca^{2+}-binding motif of CUB 26 suggesting that this CUB domain similar to CUB6 or CUB8 is important for ligand binding (**data not shown**).

Interestingly, all the patients with biallelic *CUBN* mutations showed a very similar phenotype. While no signs of vitamin B12 deficiency, such as megaloblastic anemia, could be detected, the patients shared a mild proteinuria ranging from 0.5 to 3g/24h and 0.17 to 5 g/L (**data not shown**). The proteinuria was an atypical tubular proteinuria because the proportion of albumin in the urinary protein was higher than 50% and urinary beta2-microglobulin was

low or absent (**data not shown**) [3, 15]. By contrast, beta1-microglobulin was often elevated, reflecting the affinity of cubilin for albumin and beta1- but not beta2-microglobulin. In all cases, renal function was normal at a median age of 25 years, as measured by serum creatinine levels or eGFR **data not shown**). Renal biopsies had been performed in seven cases, and in all cases lesions were minimal, unspecific or not present. Because of the familial albuminuria the suspected clinical diagnosis was SRNS or in some cases, where hematuria was also present, Alport syndrome. In six patients, treatments with ACE inhibitors had already been started but without any lowering effects on the proteinuria.

To investigate whether *CUBN* mutations that lead to proteinuria are always associated with normal renal function, we next assembled an additional cohort of 107 patients with chronic subnephrotic proteinuria ("chronic PU cohort"). For all patients, previous sequencing efforts using NGS panels containing the major SRNS genes but lacking *CUBN* had failed to identify the molecular cause of the proteinuria. In this cohort, 41 had normal renal function (serum creatinine <110 µmol/L in men or 90 µmol/L in women or eGFR > 60), whereas 30 patients had end-stage renal disease (ESRD; <10ml/min GFR, transplanted or on hemodialysis). All patients were sequenced with a custom-made NGS panel enriched for genes important for proximal tubule function, such as *CUBN* (**data not shown**). The sequencing revealed that 11.2% of the patients in this cohort have homozygous or at least two heterozygous mutations in *CUBN,* translating into a mutation rate of 29.3% in individuals with chronic proteinuria and normal renal function and 0% in patients with chronic proteinuria and reduced renal function.

The 12 patients with *CUBN* mutations had a very similar phenotype to the above described patients (Table 1), including a mild proteinuria range, no or unspecific lesions on the 6 kidney biopsies, no proteinuria lowering effects with ACE inhibitors, normal renal function, even for the oldest patient at the age of 70 years. This also explains why it was sometimes difficult to obtain follow-up clinical information for our cohort patients (**data not shown**). Of note, we also identified two patients with *CLCN5* and *OCRL1* mutations, respectively, responsible for Dent's disease 1 and 2 (**data not shown**). However, in these patients serum creatinine levels were elevated, suggesting a fundamental difference between Dent's disease and cubilin deficiency. Except for one single heterozygous mutations localized within CUB8, close to the vitamin B12/IF-binding region (**data not shown**), all identified variants from the discovery cohort were in the C-terminal CUB11-27 (**data not shown**). Structural models showed that many of the mutations could have effects on the folding and function of the CUB domains. Several mutations, such as p.G1928V and p.D3609H, localized in the vicinity of the

Ca^{2+}-binding motif of CUB13 and 26, again suggesting that these CUB domains could be important for albumin binding (**data not shown**).

Based on these data, we evaluated four low-frequency and common C-terminal variants that show strong association with albuminuria in GWAS studies [10-12, 16, 17]: p.A1690V, p.N2157D, p.A2914V and p.I2984V. According to the structural modeling, all of them have the potential to disturb CUB domain stability or ligand binding (**data not shown**). Except for A1690V, the frequencies of the GWAS variants are higher than our cut-off (f=0.002) for defining functional variants (see methods), which is why they were not included in the initial analysis (in-house genome database: f(A1690V)=0.00109, f(N2157D)=0.00667, f(A2914V)=0.00945, f(I2984V)=0.0932; gnomAD: f(A1690V)=0.00173, f(N2157D)=0.00565, f(A2914V)=0.0122, f(I2984V)=0.0875 (**data not shown**)). Unlike our novel rare clinical *CUBN* variants, these GWAS variants have been included on many SNP microarrays. To test whether the four albuminuria-associated GWAS variants affect eGFR, we performed a large meta-analysis of population-based cohorts from the CKDGen Consortium, comprising between 331,340 and 597,710 individuals. In all four cases, we found a modest but significant association with higher eGFR for the minor compared to the major allele (**data not shown**). This was also confirmed in a smaller, independent cohort comprising 13,550 individuals with and without type 2 diabetes. While p.N2157D was only rarely found in this cohort, the effect size on eGFR correlated with the effect size on albuminuria for the other three variants both in diabetic and non-diabetic individuals (**data not shown**). Together, these data provide strong support for the benign nature of the albuminuria.

Discussion

The combined analysis of the genetic kidney disease and chronic PU cohorts identified 25 patients with isolated proteinuria and normal renal function due to recessive *CUBN* variants. We further show that the high percentage of urinary albumin in these patients is often misinterpreted as glomerular injury in the clinical setting, justifying renal biopsy and ACE or AT1 receptor inhibition as proteinuria-lowering treatment. Yet, we show that the proteinuria is not associated with an unfavourable prognosis for kidney disease in our patients. Apart from establishing a diagnosis in individuals with isolated subnephrotic proteinuria, the detection of *CUBN* mutations can therefore avoid inefficient therapies aimed at reducing glomerular proteinuria.

Our data also provide strong support for the genotype-phenotype correlation associated with *CUBN* mutations [18, 19]. While all the I-GS mutations can exclusively be found before

or within vitamin B12/IF-binding region (CUB5-8), we show here that isolated proteinuria is caused by mutations located after this region. In contrast to previous *in vitro*-studies [20], this suggests that the renal ligands, most notably albumin, bind to more C-terminal CUB domains, e.g. those that possess both a Ca^{2+}-binding motif. Accordingly, missense mutations that only affect vitamin B12/IF-binding, such as the Finnish mutation p.P1297L, are typically not associated with proteinuria [21]. Although this needs to be analyzed more systematically, the reverse conclusion is that I-GS patients that have proteinuria should have mutations that either affect general expression or the interaction with amnionless or lead to cubilin truncation. An interesting observation from such I-GS patients is that their proteinuria is not associated with a loss of renal function [8], similar to the isolated proteinuria cases described here. Combined with functional studies showing that vitamin B12 uptake is maintained when the receptor is truncated after CUB8 [22] and our gnomAD analysis showing that premature truncation of cubilin is more likely to happen after CUB8, it can be concluded that in humans vitamin B12 malabsorption is less tolerated than increased albuminuria.

Our findings were supported and extended to the general population through studies of over half a million participants. By evaluating four *CUBN* variants with strong albuminuria association, we found a modest but significant association with higher eGFR. Support for any functional effects of these variants comes from our structural modelling and from the observation from our chronic PU cohort that compound heterozygosity with a low-frequency variant passing our filtering criteria can also lead to the expected phenotype of chronic proteinuria and normal renal function (**data not shown**). However, with variants identified in GWAS functional effects need to be further established through experimental research because of potential correlations of nearby SNPs. Indeed, we found that in the European population p.A2914V is in linkage disequilibrium with the more frequent p.I2984V (D'=1), suggesting that the albuminuria and eGFR association might mostly stem from p.A2914V whose effect sizes are higher than those of p.I2984V.

Nevertheless, it can be speculated that in addition to the benign nature of this kind of proteinuria, there may even be some advantage in reducing proximal tubular uptake, for example in conditions where the tubules are overloaded with proteins and lipids [23]. Support for this view comes from the clinical observation that tubular damage is key for the progression of diabetic kidney disease and primary FSGS [24, 25], from mouse models of proteinuria and hyperlipidemia [26, 27], in which the reduction of tubular uptake was shown to prevent early injury, or from mice lacking albumin, that become protected against glomerular disease [28]. As evidence for positive selection of human C-terminal *CUBN* variants has already been

demonstrated [23, 29], it would be interesting to explore in more detail what renal disease condition may have led to an enrichment of such C-terminal variants in the human genome.

In sum, our study proposes a new paradigm for the non-detrimental effects of tubular proteinuria, which contrasts the general dogma that proteinuria is always damaging. We therefore recommend genetic testing for *CUBN* variants in individuals with chronic subnephrotic proteinuria to avoid unnecessary further medical actions.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method of treating a chronic kidney disease with glomerular proteinuria in a patient in need thereof comprising administering to the patient a therapeutically effective amount of an inhibitor of cubilin.

2. The method of claim 1 wherein the inhibitor of cubilin is suitable for increasing the glomerular filtration rate of the patient.

3. The method of claim 1 wherein the inhibitor of cubilin is a small organic molecule.

4. The method of claim 1 wherein the inhibitor of cubilin is an antibody.

5. The method of claim 4 wherein the antibody is a single domain antibody.

6. The method of claim 1 wherein the inhibitor of cubilin is an inhibitor of cubilin expression.

7. The method of claim 6 wherein the inhibitor of expression is an antisense oligonucleotide, a siRNA or a ribozyme that blocks the translation of cubilin mRNA.

8. The method of claim 1 wherein the inhibitor of cubilin is administered to the patient in combination with an anti-proteinuric therapy.

9. The method of claim 8 wherein the anti-proteinuric therapy consists in administering to the patient a therapeutically effective amount of an angiotensin-converting enzyme inhibitor (ACE inhibitor) and or angiotensin receptor blocker (ARB).

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/078592

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K31/7105 A61K31/401 A61K45/06 A61P13/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, CHEM ABS Data, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MIN LI ET AL: "Silencing megalin and cubilin genes inhibits myeloma light chain endocytosis and ameliorates toxicity in human renal proximal tubule epithelial cells", AMERICAN JOURNAL OF PHYSIOLOGY: RENAL PHYSIOLOGY, vol. 295, no. 1, 1 July 2008 (2008-07-01), pages F82-F90, XP055706575, United States ISSN: 1931-857X, DOI: 10.1152/ajprenal.00091.2008	1,2,6,7
Y	the whole document	3-5,8,9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 June 2020	03/07/2020

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/078592

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	PIERRE J. VERROUST ET AL: "The tandem endocytic receptors megalin and cubilin are important proteins in renal pathology", KIDNEY INTERNATIONAL, vol. 62, no. 3, 1 September 2002 (2002-09-01), pages 745-756, XP055706160, GB ISSN: 0085-2538, DOI: 10.1046/j.1523-1755.2002.00501.x the whole document -----	1-9
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/078592

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

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PCT/EP2019/078592

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