

CUBN mutation: a benign genetic cause of proteinuria?

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Proteinuria is an important risk factor for renal and cardiovascular disease. It is associated with a risk for glomerulonephritis, chronic kidney disease, and end-stage renal disease. Therefore, if persistent proteinuria is detected, kidney biopsy is considered to diagnose and treat the underlying disease. Recently, variants in the cubilin (*CUBN*) gene, which is associated with albuminuria, have been reported. This gene encodes cubilin, a membrane glycoprotein receptor expressed in the renal proximal tubules. Cubilin is a component of the megalin and cubilin-amnionless complex that mediates albumin reabsorption into the proximal tubules through endocytosis. A defect in cubilin leads to a reduction in albumin reuptake, resulting in albumin-dominant proteinuria. Although numerous controversies exist, several reports suggest that cubilin defects lead to proteinuria with a high portion of albuminuria but may not impair renal filtration function. If albuminuria due to reduced cubilin function is confirmed as a benign condition, we can consider using genetic studies to detect *CUBN* mutations in patients with proteinuria and they may not require any treatment or kidney biopsy. Here, we review recent papers on *CUBN* mutations and discuss the prognosis and management of individuals with this mutation.

Keywords: Albuminuria; Endocytosis; Kidney tubules, proximal

Introduction

Proteinuria is a well-known risk factor for progressive renal impairment and cardiovascular disease. Kidney damage due to proteinuria is thought to be caused by progressive proliferation due to increased glomerular capillary pressure and plasma protein filtration. Therefore, if persistent proteinuria is detected, kidney biopsy is considered for the diagnosis and treatment of the underlying disease. However, for diseases in which renal damage is not caused despite the patient showing symptoms of proteinuria, there may be cases in which various treatments used for protection of renal damage are unnecessary. Recently, with the development of next generation sequencing (NGS) technology, a mutation in the cubilin (*CUBN*) gene was detected

in patients with hereditary kidney diseases that have chronic proteinuria. Although this disease leads to proteinuria, there are many questions about its progression to renal impairment. In this review, we focus on cubilin and the latest views on the prognosis of the *CUBN* mutation.

Pathophysiology of proteinuria

Proteinuria is mainly divided into glomerular proteinuria and tubular proteinuria based on the origin of proteinuria. Dysfunction of the glomerular filtration barrier results in glomerular proteinuria, mainly albumin, which has a molecular weight of approximately 67 kDa. In contrast, tubular proteinuria is caused by the dysfunction of tubular reabsorption. It is composed of

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low molecular weight proteins, mainly $\beta 2$ microglobulin and $\alpha 1$ microglobulin. Furthermore, albumin accounts for less than half of tubular proteinuria.

The passage of molecules through the glomerular basement membrane (GBM) is size- and charge-dependent. The GBM has a pore size of 4 nm and a negative charge due to the presence of glycoaminoglycans. Therefore, negatively charged and large molecules cannot pass through the GBM easily. Albumin has a negative charge and has an ellipsoid shape with a 14-nm width and 3.8-nm length. Because the length (3.8 nm) of albumin is shorter than the GBM pore size (4 nm), small amounts (22–32 mg/L) of albumin can be filtered through the GBM. The albumin is then reabsorbed at the early and late parts of the proximal tubules and descending part of the straight tubules. Finally, only approximately 100 mg of albumin is secreted per day [1,2].

Genetic aspects of proximal tubular reabsorption

Proximal tubules have receptors called megalin and cubilin-amnionless complex (CUBAM) at the brush border. These receptors bind to several ligands, including albumin, and transfer these ligands into the endosome in the proximal tubular cytoplasm via clathrin-mediated endocytosis. Subsequently, these ligands are degraded by the lysosome or undergo transcytosis via the neonatal Fc receptor [3–5].

Several genetic tubular diseases develop via the mutations in this process. Donnai-Barrow syndrome (OMIM #222448) is caused by a mutation in megalin (gene name: *LRP2*), Imerslund-Gräsbeck syndrome or megaloblastic anemia 1 (IGS or MGA1) (OMIM #261100) by a mutation in cubilin (gene name: *CUBN*), Dent disease (OMIM #300009) by a mutation in endosomal chloride voltage-gated channel 5 (gene name: *CLCN5*), Lowe syndrome (OMIM #3090000) by a mutation in endosomal inositol polyphosphate-5-phosphatase (gene name: *OCRL*), and nephrotic cystinosis (OMIM #219800) by a mutation in lysosomal cystinosis (gene name: *CTNS*) (Fig. 1) [6–11].

Molecular structures of megalin, cubilin, and amnionless

Cubilin, an albumin receptor, was first described by Birn et al. in 2000 [12]. It is an extracellular glycoprotein approximately 460 kDa in size, and the gene encoding cubilin is located on

chromosome 10p12.33–p13 in humans. Cubilin has 110 amino acid N-terminals, 8 epidermal growth factor-like domains, and 27 complement C1r/C1s, Uegf, and Bmp1 (CUB) domains (Fig. 2) [1]. Because cubilin does not have a transmembrane domain, not fully understood, it interacts with amnionless (CUBAM) for endocytosis.

Megalyn has a molecular weight of 600 kDa, and the encoding gene is located on chromosome 2q24–q31 in humans. It has large N-terminal extracellular domains, a single transmembrane domain, and a short C-terminal cytoplasmic fragment (Fig. 2) [1].

Clinical manifestations of *CUBN* mutation

Cubilin is expressed in podocytes, proximal tubules, and the small intestine. As described above, cubilin plays a role in albumin reabsorption in the proximal tubule. Furthermore, it is a receptor of the intrinsic factor-vitamin B₁₂ (IF-B₁₂) complex in the small intestine. Therefore, patients with *CUBN* mutations

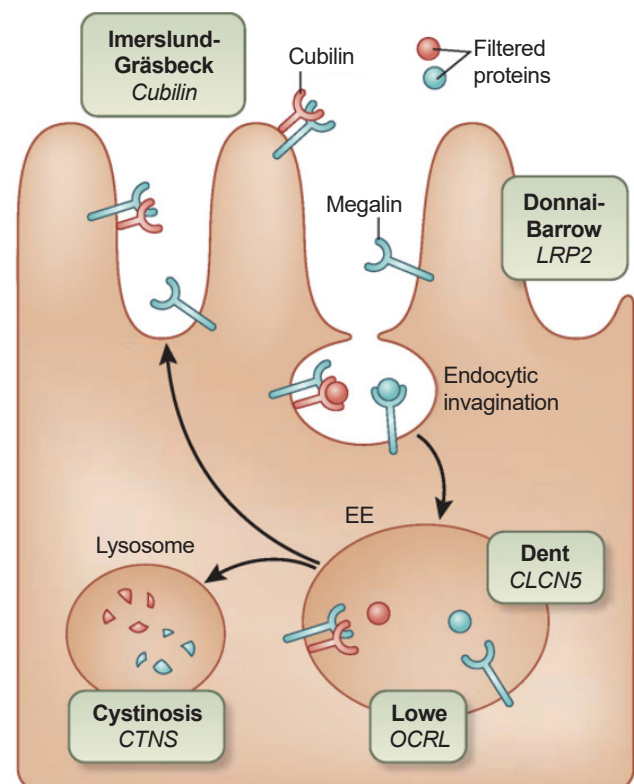


Fig. 1 Endo-lysosomal system in proximal tubular epithelial cells and the related genetic disorders. EE, early endosome. Adapted from Willnow, Kidney Int 2017;91:776–8 [6].

can develop proteinuria and/or MGA.

IGS (or MGA1), caused by mutations in *CUBN*, is a rare, autosomal recessive disorder that is characterized by selective

intestinal vitamin B₁₂ malabsorption and results in MGA. It also has low molecular weight proteinuria. In most cases, proteinuria in IGS is sub-nephrotic range proteinuria and is characterized by more than 50% albuminuria [9,10].

Fig. 3 shows the currently known loci of the *CUBN* mutation. Most mutation sites of *CUBN* in IGS are located at the N-terminal of cubilin and affect the interaction sites with amnionless or the IF-B₁₂ binding CUB domains 5–8. However, in patients with only proteinuria, almost all variants were located after the IF-B₁₂ binding domain. This suggests that C-terminal CUB domains are crucial for renal protein reabsorption [10].

The history of studies on *CUBN* mutations

Since 1999, IGS with MGA and proteinuria has been reported [13–15]. In 2011, Ovunc et al. [16] reported a homozygous frame-shift mutation in *CUBN* in two siblings from consanguineous parents with intermittent nephrotic-range proteinuria by exome sequencing. Furthermore, a meta-analysis through a genome wide association study (GWAS) in the general and diabetic population highlighted the relationship between *CUBN* single nucleotide polymorphism and proteinuria in the same year [17].

Since 2015, large-scale cohort studies for genetic diagnosis of patients with proteinuria have shown a certain number of patients with *CUBN* mutations [18–23]. One of these was the result of three large cohort studies on *CUBN* mutations by Bedin et al. in 2020 [21]. A total of 2,216 patients were enrolled in three cohort studies. In the first cohort (genetic kidney disease cohort I), NGS was performed on 759 patients whose suspected genetic causes of renal disease included steroid resistant nephrotic syndrome (SRNS), Alport syndrome (AS), nephronophthisis, congenital anomalies of kidney and urinary tract, tubulointerstitial nephritis, polycystic kidney disease, and renal tubular

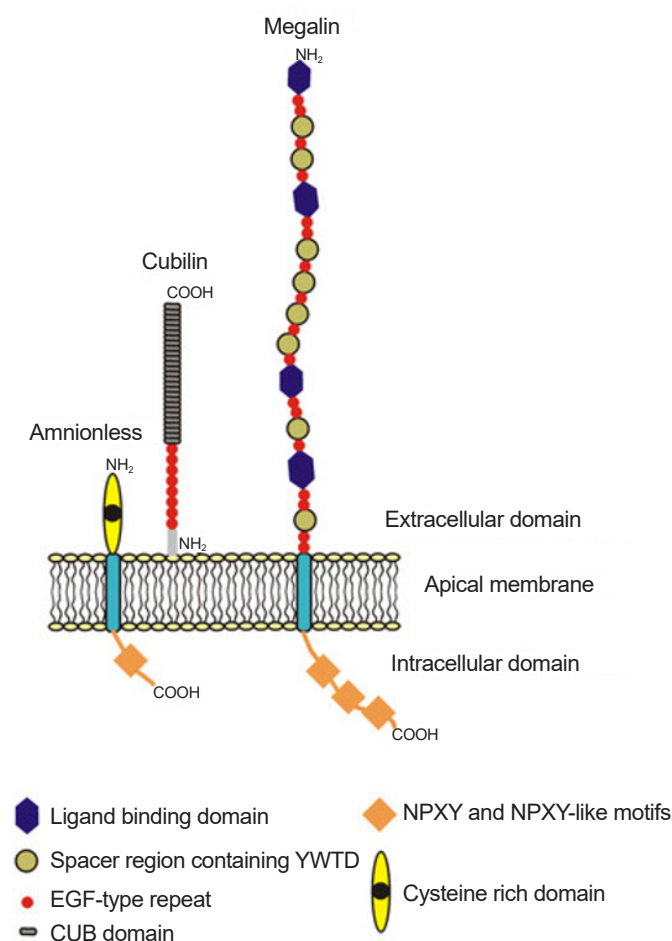


Fig. 2. Endocytic megalin and cubilin-amnionless complex in the apical membrane of the renal proximal tubule. EGF, epidermal growth factor; CUB, complement sub-components C1r/C1s, Uegf, and Bmp1. Adapted from Gburek et al. Int J Mol Sci 2021;22:5809 [1].

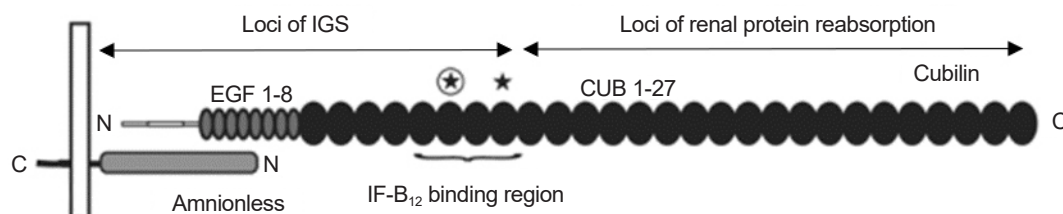


Fig. 3. Schematic representation of cubilin and amnionless. IGS, Imerslund-Gräsbeck syndrome; EGF, epidermal growth factor; CUB, complement sub-components C1r/C1s, Uegf, and Bmp1; IF-B₁₂, intrinsic factor-vitamin B₁₂. Adapted from Storm et al. BMC Med Genet 2013;14:111 [10].

disorder. For the second cohort (genetic kidney disease cohort II), NGS was performed on 1,350 patients whose suspected genetic cause of proteinuria included SRNS and AS, and for the third cohort (chronic PU cohort), NGS was performed on 107 patients with chronic proteinuria. In these three cohort studies, biallelic *CUBN* mutations were observed in 39 patients. The 39 patients with biallelic *CUBN* mutations received a diagnosis between 0 and 36.6 years and did not show MGA or renal impairment during the follow-up period (age at last follow-up: 0–71.7 years). Their proteinuria was less than 1 g/day, and the albumin content in the urine protein was more than half. Unlike other tubular proteinuria including caused by *LRP2* mutation, β_2 microglobulin was almost absent or low. Kidney biopsy was conducted on a total of 19 patients. One patient in the second cohort showed early focal segmental glomerulonephritis (FSGS), and the remaining showed no specific finding or minimal lesions. All patients with the *CUBN* mutations in the third cohort showed no protein lowering effects when treated with angiotensin converting enzyme inhibitor (ACEi). Furthermore, almost all variants of the *CUBN* gene were located after the IF-B₁₂ binding domain.

In this paper, another large-scale meta-analysis of existing GWAS was conducted with the general population. In previous studies, four C-terminal variants (p.A1690V, p.N2157D, p.A2914V, and p.I2984V) were shown to have strong associations with albuminuria using GWAS. According to structural modeling, these four GWAS variants have the potential to disturb the CUB domain stability or ligand binding. Two more meta-analyses were conducted: a large meta-analysis of the CKDGen Consortium's population-based cohorts consisting of 331,340 to 597,710 individuals, and a smaller one on an independent cohort consisting of 13,550 individuals with and without type 2 diabetes mellitus (T2DM). The results showed that the population with four C-terminal variants had a tendency of exhibiting high glomerular filtration rate (GFR), despite having albuminuria. Thus, this paper mentioned that the C-terminal *CUBN* mutation may have a renal protective effect.

In addition, in a protein-coding genetic variation analysis for 60,000 people in 2016 [24], *CUBN* and *AMN* (which encodes amnionless) showed very low loss-of-function intolerance scores. These data strongly indicate the benign nature of albuminuria associated with C-terminal *CUBN* variants.

The second large-scale study included a cohort of a Spanish group in 2021 [22]. Genetic testing was performed on 347 families with persistent proteinuria with a suspected monogenic

cause. Among them, *CUBN* biallelic mutations were observed in 15 patients in 12 families. The age at diagnosis of proteinuria ranged between 9 months and 44 years. Furthermore, 54% of urine protein was albumin. The follow-up period was 7 years on average, and no improvement in proteinuria was observed after treatment with ACEi. Among 15 biopsies, 13 were normal, but two patients had more severe phenotypes. One patient was diagnosed as SRNS with minimal change disease through the kidney biopsy. This patient used prednisolone and cyclophosphamide and showed normal kidney function and blood pressure during follow-up, but proteinuria persisted. Another patient was diagnosed as FSGS on the biopsy and showed hypertension, hyperuricemia, decreased GFR, hypertension induced cardiomyopathy, T2DM, and obesity during the follow-up period. The authors thought that the progress of these patients was due to comorbidities.

Meanwhile, Yang et al. in China [25] described three FSGS patients with *CUBN* mutations in 2022. Patients were diagnosed at the age of 6 years, 8 years, and 11 years and showed no MGA. They had mild proteinuria, and their urine protein to creatinine ratio were 0.3–0.5, β_2 microglobulin was normal or slightly high, and urine protein electrophoresis showed more than 50% albumin. The serum albumin level of the patients was normal and showed 1–2 segmental sclerosis in kidney biopsy. Two patients were accompanied by interstitial fibrosis, and a crescent was also observed in these two cases. All electron microscopy showed foot process effacements. All *CUBN* mutations were biallelic, and all were located close to the C-terminal. These three patients were administered tacrolimus after FSGS was confirmed in the biopsy, and their protein to creatinine ratios decreased slightly during the follow-up period of more than 3 months. The authors suggested that albuminuria probably originated not only from proximal tubular malabsorption but also from podocyte dysfunction in patients with *CUBN* mutations. This is because patients showed FSGS with pathological changes in podocytes and reduced proteinuria after administration of tacrolimus. In addition, one of four patients in the genetic kidney disease cohort II with *CUBN* mutations in the study of Bedin et al. also showed FSGS [21]. Furthermore, some studies showed that cubilin is expressed in rat and human podocytes and mediates albumin endocytosis in human podocytes. Another line of evidence suggests that cubilin has a higher binding affinity for albumin compared to that for megalin. Megalin can function as a sensor of albumin to affect cell survival via the phospho-

Table 1. History of reports on *CUBN* mutation

Author (year)	Study group, design	No. of patients	Summary	Age at diagnosis (yr)	Age at last follow-up (yr)	Abnormal biopsy findings/total biopsy numbers	Impaired kidney function
Ovunc et al. (2011) [16]	USA, case report	2	A homozygous frameshift mutation in <i>CUBN</i> gene in two siblings of consanguineous parents with intermittent nephrotic-range proteinuria	4/5	ND	Not done	No
Jayasinghe et al. (2019) [26]	Australia, case report	2	Long continuous stretches of homozygosity representing ~4.5% of the genome (implicated a homozygous stop-gain variant in <i>CUBN</i> (10p12.31)) in two siblings with a consanguineous family history with incidental isolated proteinuria	4/8	ND	Normal LM and global GBM thinning on EM/1	No
Sadowski et al. (2015) [18]	International cohort	5	<i>CUBN</i> mutations: 5/2,016 individuals (0.28%) in an international SRNS cohort of 1,783 families	4.8/6/11.9/ND/ND	ND	2 FSGS/ND	ND
Schapiro et al. (2019) [19]	International cohort	3 (1 family)	<i>CUBN</i> mutations: 1/362 families (3/371 individuals) with proteinuria and hematuria with an onset before 25 yr of age	ND	ND	ND	ND
Nagano et al. (2020) [20]	Japanese cohort	1	<i>CUBN</i> mutations: 1/230 patients with severe proteinuria	3	ND	Minor glomerular abnormality	No
Bedin et al. (2020) [21]	European cohort	39	<i>CUBN</i> mutations: 39 patients in three cohorts (genetic studies of 2,216 patients with chronic proteinuria)	0–36.59	0–71/17	1 FSGS/19	No
Domingo-Gallego et al. (2021) [22]	Spanish cohort	15	<i>CUBN</i> mutations: 15 patients (12 families)/347 families with persistent proteinuria suspected of monogenic cause	Median age 4 (0.8–44)	Follow-up duration 7 (2–39)	1 FSGS, 1 MCD/15	1 (FSGS)
Yang et al. (2022) [25]	China, case report	3	3 Patients with <i>CUBN</i> biallelic pathogenic variants presented as isolated proteinuria and FSGS in kidney biopsy	6/8/11	ND	3 FSGS/3	No
Cicek et al. (2022) [23]	Turkey, case series	6	<i>CUBN</i> mutations: 6 patients (3.8%)/158 patients with SRNS or chronic proteinuria	Median age 7.3 (4.7–11.7)	5.1–20.4	1 FSGS/4	No
Gan et al. (2022) [27]	China, case series	2 Families	Compound heterozygous variants in <i>CUBN</i> in 2 families	8/ND	ND	2 Normal/2	No

CUBN, cubulin; LM, light microscopy; GBM, glomerular basement membrane; EM, electron microscopy; SRNS, steroid resistant nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease; ND, no data.

inositolide 3-kinase/protein kinase B pathway. If a large quantity of albumin binds to megalin due to cubilin dysfunction, it can promote cell apoptosis [28-30]. Furthermore, patients with FSGS had at least one relatively serious mutation in one allele. These include nonsense mutations, insertions, deletions, or mutations in splice sites which led to frameshift mutations or protein truncation.

The most recent report on *CUBN* mutations was a cohort study by a group in Turkey published in 2022 [23]. Through genetic tests performed on 158 patients with SRNS or chronic proteinuria, the authors found a total of six *CUBN* mutations, all of which were C-terminal. Kidney biopsy was performed in four of six patients, one of whom was diagnosed with FSGS, and a homozygous nonsense mutation was observed in this patient. ACEi or angiotensin II receptor blocker (ARB) had no therapeutic effects on all patients, and FSGS patients were also unresponsive to prednisolone, cyclosporine, and rituximab treatment. All patients showed normal renal function during follow-up even after all treatments were stopped.

Is proteinuria caused by *CUBN* mutation benign?

Table 1 shows a summary of studies on *CUBN* mutations. There is a limitation that the total number of patients with *CUBN* mutations and the follow-up duration was not sufficient to conclude that the *CUBN* mutation is benign and does not progress to chronic kidney disease (CKD) based on these studies alone. However, no patients progressed to CKD except for one patient with renal impairment due to comorbidity in these studies [22]. In addition, some reports suggested the hypothesis that the *CUBN* mutation could be benign [21,24]. The hypothesis about the reno-protective effect of *CUBN* mutation like sodium glucose cotransporter 2 inhibitor provided hope that the *CUBN* mutation could be benign [31]. However, patients exhibit FSGS on histological examination (Table 1) [18,21,23-25] and cubilin is expressed not only in renal tubules, but also in podocytes [28,29]; thus, concluding that *CUBN* mutation is benign is a challenging task. Future studies will have to be conducted with long-term follow-up of more patients for more concrete evidence.

When do we suspect *CUBN* mutation and what genetic tests should we run?

In patients with asymptomatic proteinuria who do not have hypertension, hypoalbuminemia, if the proteinuria is in the sub-nephrotic range, β_2 microglobulin is low, and albuminuria is greater than 50%, *CUBN* mutation may be suspected. If *CUBN* mutation is suspected, genetic kidney disease screening for *CUBN* mutation by whole exome sequencing or a kidney disease targeted gene panel test that includes *CUBN* is recommended prior to kidney biopsy.

Conclusion

Proteinuria, due to *CUBN* mutation, is currently presumed to be caused by kidney tubular dysfunction, which leads to chronic sub-nephrotic proteinuria with high urine albumin ratios. Therefore, if a patient with chronic sub-nephrotic proteinuria had high urine protein to creatinine ratios and normal kidney function, genetic analysis for the *CUBN* mutation must be considered. To date, there is no clear verification that patients with the *CUBN* mutation do not need ACEi or ARB for kidney protection because it is unclear if it is a benign disease. However, if a *CUBN* mutation is diagnosed, at least kidney biopsy and the use of immunosuppressants can be avoided. Future studies must be conducted to conclude whether this disease shows a benign course through long-term follow-up.

Conflicts of interest

Hyun Kyung Lee is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contributions

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