Pediatric Nephrology
https://doi.org/10.1007/s00467-022-05730-y

BRIEF REPORT

Clinical and genetic characterization of children with cubilin variants

Neslihan Cicek[1] · Harika Alpay[1] · Sercin Guven[1] · Ceren Alavanda[2] · Özde Nisa Türkkan[1] · Serim Pul[1] · Ece Demirci[1] · Nurdan Yıldız[1] · Pınar Ata[2] · Ibrahim Gokce[1]

Received: 22 April 2022 / Revised: 28 August 2022 / Accepted: 29 August 2022

Abstract

Background Cubilin is one of the receptor proteins responsible for reabsorption of albumin in proximal tubules and is encoded by the *CUBN* gene. We aimed to evaluate clinical and genetic characterization of six patients with proteinuria who had *CUBN* mutations.

Methods Patients' characteristics, serum creatinine, albumin, vitamin B_{12} levels, urine analysis, spot urine protein/creatinine, microalbumin/creatinine, beta-2 microglobulin/creatinine ratios, estimated glomerular filtration rates (eGFR), treatments, kidney biopsies, and genetic analyses were evaluated.

Results Six patients (2 female, 4 male) with an incidental finding of proteinuria were evaluated. Mean admission age and follow-up time were 7.3 ± 2.9 and 6.5 ± 5.6 years, respectively. Serum albumin, creatinine, and eGFR were normal; urine analysis revealed no hematuria, and C3, C4, ANA, and anti-DNA were negative; kidney ultrasonography was normal for all patients. Urine protein/creatinine was 0.9 ± 0.3 mg/mg, and microalbumin was high in all patients. Serum vitamin B_{12} was low in two patients and normal in four. Kidney biopsy was performed in four patients, three demonstrated normal light microscopy, and there was one focal segmental glomerulosclerosis (FSGS). Genetic tests revealed four homozygous and two compound heterozygous mutations in the C-terminal part of cubilin. All patients had normal eGFR and still had non-nephrotic range proteinuria at last visit.

Conclusions *CUBN* gene mutations should be considered in patients with isolated non-nephrotic range proteinuria and normal kidney function. Diagnosing these patients, who are thought to have a better prognosis, is important in terms of avoiding unnecessary treatment and predicting prognosis. *CUBN* gene mutations may also present as FSGS which extends the spectrum of renal manifestation of these patients.

Keywords Children · Cubilin · Focal segmental glomerulosclerosis · Proteinuria

Introduction

Proteinuria is an important risk factor that is associated with poor kidney outcome [1]. In normal conditions, a small amount of albumin is filtered through the glomeruli and reabsorbed at the proximal tubules by receptor-mediated endocytosis. Two interacting receptors, megalin and cubilin, which form a complex with amnionless, are responsible for this process. Cubilin has the major role in reabsorption and is encoded by the *CUBN* gene [2, 3]. This gene is also known as the cause of Imerslund–Gräsbeck syndrome (IGS) characterized by malabsorption of vitamin B_{12} resulting in megaloblastic anemia and frequently proteinuria that is not in nephrotic range [4, 5]. Most pathogenic *CUBN* variants in IGS are found in the N-terminal half of cubilin, whereas mutations in C-terminal sections have recently been reported in patients with isolated proteinuria [6, 7]. In the present study, we evaluated six patients with isolated proteinuria from unrelated families with mutations in the *CUBN* gene.

✉ Neslihan Cicek
drneslihancicek@yahoo.com

[1] Department of Pediatric Nephrology, Marmara University School of Medicine, Fevzi Çakmak Mahallesi Muhsin Yazıcıoğlu Caddesi No: 10, Ust Kaynarca/Pendik, Istanbul, Turkey

[2] Department of Medical Genetics, Marmara University School of Medicine, Fevzi Çakmak Mahallesi Muhsin Yazıcıoğlu Caddesi No: 10, Ust Kaynarca/Pendik, Istanbul, Turkey

Published online: 16 September 2022

Methods

Clinical data were retrospectively obtained from medical records. Patients' demographic characteristics, serum creatinine, albumin, vitamin B_{12} levels, urine analysis, spot urine protein/creatinine, microalbumin/creatinine, and beta-2 microglobulin/creatinine ratios at presentation and at last visit, as well as estimated glomerular filtration rates (eGFR) were evaluated (Table 1). eGFR was calculated by Schwartz formula with a constant $k = 0.55$ for children and adolescent girls and $k = 0.7$ for adolescent boys since serum creatinine was measured with Jaffe method, and eGFR ≥ 90 mL/min/1.73 m^2 was defined as normal [8]. Kidney biopsy results and treatment modalities were also reviewed. We performed genetic analysis in 158 proteinuric patients with steroid resistant nephrotic syndrome or chronic proteinuria. Proteinuria was defined as urine protein/creatinine ratio (u pr/cr) > 0.2 mg/mg and nephrotic range proteinuria as > 2 mg/mg in spot urine. Microalbuminuria was defined as urine microalbumin/creatinine ratio (u ma/cr) > 30 mg/gr and beta-2 microglobulinuria as urine beta-2 microglobulin/creatinine (u beta-2 mg/cr) > 300 mcg/gr in spot urine.

Genetic analysis

Written informed consent was obtained from all patients after clinical histories and detailed pedigree analyses were performed. DNA isolation from peripheral blood was performed with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Forty-four genes related to nephrological disorders including the *CUBN* gene (NM_001081) were sequenced using Sophia Nephropathies Solution (NES) kit via Illumina Nextseq 500 (San Diego, CA, USA). Sophia DDM-V4® platform was used for data analysis. In population studies such as ExAC, ESP, 1000 Genome (1000G), and Genome aggregation database (gnomAD), variants with minor allele frequency (MAF) < 0.01 were filtered out. Retained variants which related to patient's clinical findings were searched in Clinvar and Human Gene Mutation Database (HGMD). Detected variants were classified according to ACMG criteria. Segregation analyses were performed via Illumina Miseq (San Diego, CA, USA).

Results

Six patients (2 female, 4 male) were evaluated with a mean admission age of 7.3 ± 2.9 (4.7–11.7) years and a follow-up time of 6.5 ± 5.6 (0.4–14.7) years. Four patients were referred to our center from other hospitals and two were admitted to our hospital. All patients were referred to the Pediatric Nephrology Department with an incidental finding of persistent proteinuria during investigation of abdominal pain, fever or loss of appetite. Serum albumin, creatinine, eGFR were all in normal ranges, urine analysis revealed no hematuria at admission and at last visit, and C3, C4, ANA, anti-DNA were negative for all patients. Urine pr/cr at admission was 0.9 ± 0.3 mg/mg (0.6–1.3), not exceeding 1 g/day (250–721 mg/day), u ma/cr was high in all patients with a mean of 472.8 ± 231.2 mg/gr (298–860) and u pr/cr at last visit was 0.7 ± 0.2 mg/mg (0.4–0.9). Serum vitamin B12 was normal in four patients, slightly low in two patients and increased to normal ranges after oral vitamin B12 treatment with a dosage of 1 mg/day, which excludes Imerslund-Gräsbeck syndrome which does not respond to

Table 1 Clinical and laboratory data of patients with CUBN variants

Patients	Pt-1	Pt-2	Pt-3	Pt-4	Pt-5	Pt-6
Age ad (years)	11.7	10.1	5.7	6.5	5.1	4.7
Gender	F	M	M	M	M	F
Follow-up time	2.7	4.6	14.7	4.2	12.1	0.4
Albumin ad/al (gr/L)	45/42	47/44	47/49	48/43	53/54	50/50
eGFR ad/al (ml/min/1.73 m^2)	172.4/152.1	202.1/175.8	151.9/144.3	234.6/155.4	173.5/177.6	194.4/158.7
u pr/cr ad/al (mg/mg)	1.1/0.8	0.6/0.6	0.6/0.4	1.1/0.6	1.3/0.7	0.7/0.9
u ma/cr ad/al (mg/gr)	625/298	298/344	287/312	302/457	465/346	860/622
u beta-2 mg/cr al (microgr/gr)	122	185	42.5	57.6	19.4	63.8
Hb (gr/dl)	11	12.7	12.9	11.6	12.1	12.4
Serum vitamin B12	390(N)	147(L)	397(N)	620(N)	213(L)	556(N)
Kidney biopsy	Normal	Normal	Normal	–	FSGS	–

ad at diagnosis, *al* at last visit, *cr* creatinine, *eGFR* estimated glomerular filtration rate, *gr* gram, *Hb* hemoglobin, *L* low, *ma* microalbumin, *mg* microglobulin, *N* normal, *pr* protein, *Pt* patient, *u* urine

Proteinuria is defined as urine protein/creatinine ratio (u pr/cr) > 0.2 mg/mg, microalbuminuria as urine microglobulin/creatinine ratio (u ma/cr) > 30 mg/gr, and beta-2 microglobulinuria as urine beta-2 microglobulin/creatinine ratio (u beta-2 mg/cr) > 300 microgr/gr

oral treatment. Kidney ultrasonography was normal in all patients. Kidney biopsy was performed in four patients and demonstrated normal light microscopy and negative immunofluorescence examination in three patients. Podocyte foot processes were well preserved on electron microscopy in these patients. Patient 5 with the highest proteinuria had two biopsies performed 3 years apart. The first biopsy revealed one global sclerosis in 50 glomeruli, and the second biopsy revealed one periglomerular fibrosis in 27 glomeruli. Immunofluorescence examination was negative, and there was obliteration in epithelial cells on electron microscopy. He was diagnosed with focal segmental glomerulosclerosis (FSGS) with these histopathological findings. Two patients had ramipril (1.6–2 mg/m^2/day), one patient had enalapril (0.3–0.5 mg/kg/day), and two patients had enalapril and losartan (0.5 mg/kg/day) together as treatment, whereas patient 5 also had corticosteroid, ciclosporin, and rituximab treatments. We followed up our last patient (patient 6), who was diagnosed recently, with no treatment. ACEi/ARBs were discontinued in five patients and there was no increase in proteinuria after 3 months of drug cessation. At last visit, all patients had non-nephrotic range proteinuria and normal kidney functions.

When available, genetic analysis was performed for all six patients in $5.4^{t} \pm 5.3$ (0.4–13.3) years of follow-up. Genetic analysis was performed in 158 proteinuric patients with steroid resistant nephrotic syndrome or chronic proteinuria, and the percentage of *CUBN* variants in this cohort was 3.8%. Seven different (two truncating and five non-truncating) *CUBN* gene variants were detected in six patients (Table 2). Two patients were compound heterozygous, and four patients were homozygous for detected variants. All variants were localized to the C-terminal of the cubilin protein (Fig. 1).

Discussion

In the current study, six patients who presented with isolated non-nephrotic range proteinuria and normal kidney function were examined. The biopsies of four patients were normal except one with FSGS. The high proportion of microalbuminuria was interpreted as glomerular injury and ACEI/ARBs were used as proteinuria-lowering treatments, even immunosuppressive drugs in one patient who was diagnosed with FSGS. Seven different variants, all localized at the C-terminal part of cubilin, were detected in our patients. There was no increase in proteinuria after cessation of ACEI/ARBs and no deterioration in kidney functions during follow-up.

In both animal and human studies, cubilin has been found to have an essential role in the reuptake of albumin by endocytosis in the proximal tubule [2, 3, 9]. On the other hand, cubilin has also been found to be expressed by human podocytes, and it is suggested that *CUBN* gene mutations

Table 2 Genetic results of patients with *CUBN* (NM_001081.4) variants

Patients	Status	Genetic time (years)	Mutation	Position	Type of mutation	ACMG	Father	Mother
1	Compound heterozygous	1.69	c.9053A > C (p.Tyr3018Ser), c.9922 T > C (p.Trp3308Arg)	Exon 57 Exon 62	Missense Missense	Likely pathogenic Likely Pathogenic	Heterozygous C9922T > C (p.Trp3308Arg)	Heterozygous c.9053A > C (p.Tyr3018Ser)
2	Homozygous	2.93	c.10102A > G (p.Met3368Val)	Exon 63	Missense	VUS	Heterozygous c.10102A > G (p.Met3368Val)	Heterozygous c.10102A > G (p.Met3368Val)
3	Homozygous	13.30	c.10102A > G (p.Met3368Val)	Exon 63	Missense	VUS	N/A	N/A
4	Homozygous	3.01	c.8071G > A (p.Gly2691Arg)	Exon 52	Missense	VUS	Heterozygous c.8071G > A (p.Gly2691Arg)	Heterozygous c.8071G > A (p.Gly2691Arg)
5	Homozygous	11.08	**c.8317C > T (p.Gln2773*)**	Exon 53	Nonsense	Pathogenic	Exitus	Heterozygous c.8317C > T (p.Gln2773*)
6	Compound heterozygous	0.38	**c.6204C > G p.(Tyr2068*)**, c.10102A > G p.(Met3368Val), **c.4610C > T p.(Ser1537Phe)**	Exon 41 Exon 63 Exon 31	Nonsense Missense Missense	Pathogenic VUS VUS	N/A	N/A

NA not available; novel variants are shown in bold. *VUS* variant of unknown significance

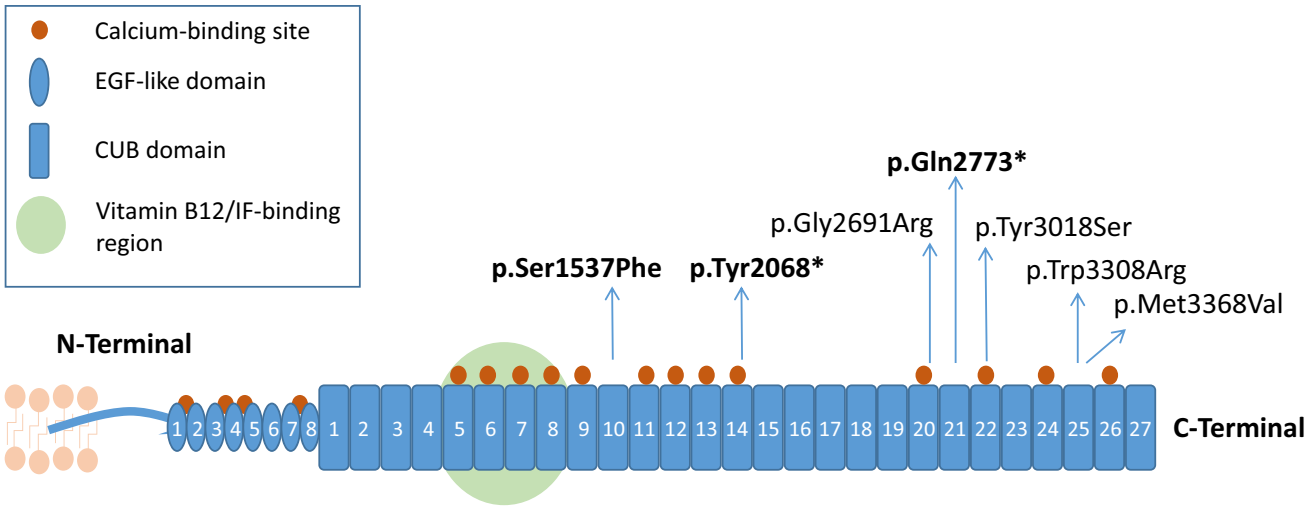

Fig. 1 Structure of cubilin protein and position of detected variants in this study. The cubilin protein consists of 8 EGF-like domains, 27 CUB domains, 17 calcium-binding sites, and one vitamin B_{12}/IF-binding region. Novel variants are shown in bold

may also lead to the dysfunction of albumin endocytosis in podocytes [10, 11].

The first cases were described by Ovunc et al. and were two siblings of consanguineous parents with isolated proteinuria and normal kidney functions. The biopsy of the first sibling was normal and they identified a homozygous variant in the *CUBN* gene in both siblings [12]. Jayahinge et al. reported a third case who was an 8-year-old boy with a homozygous *CUBN* variant with a normal kidney biopsy and normal kidney function [13]. In their cohort with 39 patients with isolated proteinuria, Bedin et al. reported that C-terminal missense variants were associated with albuminuria and increased eGFR [7]. Patients above 40 years of age with variants in the *CUBN* gene who have chronic non-nephrotic proteinuria and normal kidney functions add further evidence about the favorable prognosis [14]. All these reports suggest that proteinuria due to *CUBN* mutations could be a benign condition that may not require any proteinuria-lowering treatment or kidney biopsy [7, 12, 13].

In our cohort, all six patients who presented with isolated proteinuria had biallelic variants in the C-terminal half of cubilin and slightly high eGFR. The *c.10102A > G (p.Met3368Val)* variant in 5/12 alleles indicated that this variant could be a founder variant in the Turkish population. The rarity of this variant in large patient studies published from different countries strengthens this finding [7, 14]. ACEI/ARB treatments were discontinued and there was no increase in proteinuria at the third month. Patient 3 and patient 5 had a follow-up time over 10 years, which strengthens the evidence for favorable outcome in these patients.

Kidney biopsy has been performed in only a few patients with *CUBN* mutations due to the infrequency of cases [7, 14, 15]. When kidney biopsies of 19 patients with *CUBN* gene variants were evaluated retrospectively, one was diagnosed with FSGS and others with minimal change or no lesions [7]. In another study, three pediatric patients with isolated proteinuria, normal kidney functions, and a kidney biopsy revealing FSGS demonstrated at least a significant variant in one allele which led to frameshift or protein truncation in the *CUBN* gene [15]. One of our patients (patient 5) with a nonsense homozygous variant leading to protein truncation was also diagnosed with FSGS on kidney biopsy, and he is still on follow-up with moderate proteinuria and normal kidney function for 12 years. There was no progression in his second biopsy with presence of only one partially sclerosed glomerulus (out of 27) which could also be interpreted as near to normal pathological findings.

In conclusion, despite the limited number of patients, our results confirm that biallelic C-terminal variants in the *CUBN* gene may cause a benign condition characterized by isolated proteinuria and normal kidney function and sometimes these variants may also present with FSGS on kidney biopsy. We recommend genetic testing for *CUBN* variants in patients with isolated non-nephrotic range proteinuria and normal kidney function to avoid unnecessary treatments. Further studies with long term follow-up in patients with *CUBN* gene mutations are needed to understand more about the prognosis of these patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00467-022-05730-y.

Author contribution N Çiçek and H Alpay participated in the study design. N Çiçek, N Yildiz and S Guven analyzed and interpreted the data. O Turkkan, S Pul, E Demirci collected the clinical and laboratory data. C Alavanda and P Ata analyzed and interpreted the genetic data.

N Çiçek and H Alpay drafted the manuscript, and N Çiçek, I Gokce, N Yildiz and H Alpay reviewed the manuscript.

Each author contributed important intellectual content during manuscript drafting or revision and approved the final version.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. Hemmelgarn BR, Manns BJ, Lloyd A, James MT, Klarenbach S, Quinn RR, Wiebe N, Tonelli M; Alberta Kidney Disease Network (2010) Relation between kidney function, proteinuria, and adverse outcomes. JAMA 303:423–429. https://doi.org/10.1001/jama.2010.39
2. Amsellem S, Gburek J, Hamard G, Nielsen R, Willnow TE, Devuyst O, Nexo E, Verroust PJ, Christensen EI, Kozyraki R (2010) Cubilin is essential for albumin reabsorption in the renal proximal tubule. J Am Soc Nephrol 21:1859–1867. https://doi.org/10.1681/ASN.2010050492
3. Birn H, Fyfe JC, Jacobsen C, Mounier F, Verroust PJ, Orskov H, Willnow TE, Moestrup SK, Christensen EI (2000) Cubilin is an albumin binding protein important for renal tubular albumin reabsorption. J Clin Invest 105:1353–1361. https://doi.org/10.1172/JCI8862
4. Aminoff M, Carter JE, Chadwick RB, Johnson C, Grasbeck R, Abdelaal MA, Broch H, Jenner LB, Verroust PJ, Moestrup SK, de la Chapelle A, Krahe R (1999) Mutations in CUBN, encoding the intrinsic factor-vitamin B12 receptor, cubilin, cause hereditary megaloblastic anaemia 1. Nat Genet 21:309–313. https://doi.org/10.1038/6831
5. Ercan Z, Demir ME, Ulas T, Ingec M, Horoz M (2013) A long-term follow-up of an Imerslund Grasbeck syndrome patient with proteinuria. Nefrologia 33:147–148. https://doi.org/10.3265/Nefrologia.pre2012.Aug.11661
6. O'Toole JF, Sedor JR (2011) Are cubilin (CUBN) variants at the heart of urinary albumin excretion? J Am Soc Nephrol 22:404–406. https://doi.org/10.1681/ASN.2011010097
7. Bedin M, Boyer O, Servais A, Li Y, Villoing-Gaudé L, Tête MJ, Cambier A, Hogan J, Baudouin V, Krid S, Bensman A, Lammens F, Louillet F, Ranchin B, Vigneau C, Bouteau I, Isnard-Bagnis C, Mache CJ, Schäfer T, Pape L, Gödel M, Huber TB, Benz M, Klaus G, Hansen M, Latta K, Gribouval O, Morinière V, Tournant C, Grohmann M, Kuhn E, Wagner T, Bole-Feysot C, Jabot-Hanin F, Nitschké P, Ahluwalia TS, Köttgen A, Andersen CBF, Bergmann C, Antignac C, Simons M (2020) Human C-terminal CUBN variants associate with chronic proteinuria and normal renal function. J Clin Invest 130:335–344. https://doi.org/10.1172/JCI129937
8. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL (2009) New equations to estimate GFR in children with CKD. J Am Soc Nephrol 20:629–637. https://doi.org/10.1681/ASN.2008030287
9. Boger CA, Chen MH, Tin A, Olden M, Köttgen A, de Boer IH, Fuchsberger C, O'Seaghdha CM, Pattaro C, Teumer A, Liu CT, Glazer NL, Li M, O'Connell JR, Tanaka T, Peralta CA, Kutalik Z, Luan J, Zhao JH, Hwang SJ, Akylbekova E, Kramer H, van der Harst P, Smith AV, Lohman K, de Andrade M, Hayward C, Kollerits B, Tönjes A, Aspelund T, Ingelsson E, Eiriksdottir G, Launer LJ, Harris TB, Shuldiner AR, Mitchell BD, Arking DE, Franceschini N, Boerwinkle E, Egan J, Hernandez D, Reilly M, Townsend RR, Lumley T, Siscovick DS, Psaty BM, Kestenbaum B, Haritunians T, Bergmann S, Vollenweider P, Waeber G, Mooser V, Waterworth D, Johnson AD, Florez JC, Meigs JB, Lu X, Turner ST, Atkinson EJ, Leak TS, Aasarød K, Skorpen F, Syvänen AC, Illig T, Baumert J, Koenig W, Krämer BK, Devuyst O, Mychaleckyj JC, Minelli C, Bakker SJ, Kedenko L, Paulweber B, Coassin S, Endlich K, Kroemer HK, Biffar R, Stracke S, Völzke H, Stumvoll M, Mägi R, Campbell H, Vitart V, Hastie ND, Gudnason V, Kardia SL, Liu Y, Polasek O, Curhan G, Kronenberg F, Prokopenko I, Rudan I, Arnlöv J, Hallan S, Navis G; CKDGen Consortium, Parsa A, Ferrucci L, Coresh J, Shlipak MG, Bull SB, Paterson NJ, Wichmann HE, Wareham NJ, Loos RJ, Rotter JI, Pramstaller PP, Cupples LA, Beckmann JS, Yang Q, Heid IM, Rettig R, Dreisbach AW, Bochud M, Fox CS, Kao WH (2011) CUBN is a gene locus for albuminuria. J Am Soc Nephrol 22:555–570. https://doi.org/10.1681/ASN.2010060598
10. Prabakaran T, Christensen EI, Nielsen R, Verroust PJ (2012) Cubilin is expressed in rat and human glomerular podocytes. Nephrol Dial Transplant 27:3156–3159. https://doi.org/10.1093/ndt/gfr794
11. Gianesello L, Priante G, Ceol M, Radu CM, Saleem MA, Simioni P, Terrin L, Anglani F, Del Prete D (2017) Albumin uptake in human podocytes: a possible role for the cubilin-amnionless (CUBAM) complex. Sci Rep 7:13705. https://doi.org/10.1038/s41598-017-13789-z
12. Ovunc B, Otto EA, Vega-Warner V, Saisawat P, Ashraf S, Ramaswami G, Fathy HM, Schoeb D, Chernin G, Lyons RH, Yilmaz E, Hildebrandt F (2011) Exome sequencing reveals cubilin mutation as a single-gene cause of proteinuria. J Am Soc Nephrol 22:1815–1820. https://doi.org/10.1681/ASN.2011040337
13. Jayasinghe K, White SM, Kerr PG, MacGregor D, Stark Z, Wilkins E, Simons C, Mallett A, Quinlan C (2019) Isolated proteinuria due to CUBN homozygous mutation - challenging the investigative paradigm. BMC Nephrol 20:330. https://doi.org/10.1186/s12882-019-1474-z
14. Domingo-Gallego A, Pybus M, Madariaga L, Piñero-Fernández JA, González-Pastor S, López-González M, Simarro-Rueda E, Quintanilla-Mata ML, Matoses-Ruipérez ML, Ejarque-Vila L, Gall EC, Guirado L, Torra R, Ariceta G, Ars E (2021) Clinical and genetic characterization of a cohort of proteinuric patients with biallelic CUBN variants. Nephrol Dial Transplant. https://doi.org/10.1093/ndt/gfab285
15. Yang J, Xu Y, Deng L, Zhou L, Qiu L, Zhang Y, Zhou J (2022) CUBN gene mutations may cause focal segmental glomerulosclerosis (FSGS) in children. BMC Nephrol 23:15. https://doi.org/10.1186/s12882-021-02654-x