Clinical and genetic characterization of a cohort of proteinuric patients with biallelic CUBN variants

Andrea Domingo-Gallego¹,², Marc Pybus ¹,², Leire Madariaga ³, Juan Alberto Piñero-Fernández ⁴, Sara González-Pastor ⁵, Mercedes López-González ⁶, Esther Simarro-Rueda ⁷, María Luisa Quintanilla-Mata ⁷, María Luisa Matoses-Ruipérez ⁸, Laia Ejarque-Vila ¹, Emilie Cornec-Le Gall ⁹, Lluís Guirado ², Roser Torra ¹,², Gema Ariceta ⁶, ⁷ and Elisabet Ars ¹,², ⁷

¹Molecular Biology Laboratory, Fundació Puigvert, Institut de Investigacions Biomèdiques Sant Pau (IIB-Sant Pau), Universitat Autònoma de Barcelona, REDinREN, Barcelona, Catalunya, Spain, ²Nephrology Department, Fundació Puigvert, Institut de Investigacions Biomèdiques Sant Pau (IIB-Sant Pau), Universitat Autònoma de Barcelona, Medicine Department, REDinREN, Barcelona, Catalunya, Spain, ³Pediatric Nephrology Department, Hospital Universitario Cruces, Instituto de Investigación Sanitaria Biocruces-Bizkaia, CIBERER, CIBERDEM, Universidad del Pais Vasco UPV/EHU, Barakaldo, Spain, ⁴Nephrology Department, Pediatrics Service, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, ⁵Pediatric Nephrology Department, Hospital Universitario Germans Trias i Pujol, Barcelona, Catalonia, Spain, ⁶Pediatric Nephrology Department, Hospital Vall d’Hebron, Universitat Autònoma de Barcelona, Catalonia, Spain, ⁷Clinical Analysis Department, Hospital General Universitario de Albacete, Castilla-La Mancha, Spain, ⁸Pediatric Nephrology Department, Hospital La Fe, Valencia, Valencia, Spain and ⁹Service de Néphrologie, Hémodialyse et Transplantation Rénale, Centre Hospitalier Universitaire, Brest, France; UMR1078 Génétique, Génomique Fonctionnelle et Biotechnologies, INSERM, Université de Brest, Brest, France

∗These authors contributed equally.
Correspondence to: Elisabet Ars; E-mail: ears@fundacio-puigvert.es

GRAPHICAL ABSTRACT

Prospective cohort

Clinical and genetic characterization of proteinuric patients with biallelic C-terminal cubulin variants

| Background | C-terminal cubulin [CUBN] variants have been associated with isolated proteinuria without progression of kidney disease in children and young adults |
| Methods | Multicenter study | 347 families with proteinuria |
| Genetic testing | of families with persistent proteinuria of suspected monogenic cause | 15 patients (9 male, 6 females) from 12 families with CUBN biallelic variants |
| Families with CUBN biallelic proteinuria variants studied | Clinical, genetic laboratory and pathology data | Outcome recording |
| Results | Median age at diagnosis | 4 years (range 0.8–44) |
| | Proteinuria as incidental finding | 80% (12/15 cases) |
| | Moderate-severe proteinuria in 87% cases | 0.83 mg/mg (range 0.50–1.80) |
| | Follow-up duration | 7 years (range 2–39) |
| | Treatment with ACE/ARB in | 80% (13/15 patients) |
| | No proteinuria response in any similar proteinuria at last follow-up | 0.75 mg/mg (range 0.10–2.10) |

Renal function, serum albumin and renal pathology were normal in 13/15 patients. Two of 15 patients had an atypical, more severe phenotype, likely explained by associated comorbidities.

Conclusion

Identification of C-terminal pathogenic CUBN variants is diagnostic of an entity characterized by glomerular proteinuria, normal kidney histology, and lack of response to ACE/ARB treatment.
INTRODUCTION

Proteinuria is a well-known risk factor for progressive kidney impairment. Persistent proteinuria can be classified as glomerular, tubular or overflow. Glomerular proteinuria occurs due to increased permeability caused by anomalies in the glomerular filtration barrier, resulting in urinary loss of proteins such as albumin and immunoglobulins, as is seen in nephrotic syndrome or Alport syndrome. Tubular proteinuria occurs when there is defective reabsorption in the proximal tubules of the freely filtered low-molecular-weight (LMW) proteins. A classic example is Dent disease, which is characterized by increased LMW proteinuria but normal or low albuminuria at diagnosis. Finally, overflow proteinuria is very rarely seen in children and is mostly associated with malignant diseases [1].

In healthy humans, a small amount of albumin is filtered through the glomeruli and reabsorbed later at the proximal tubules. This process is driven by receptor-mediated endocytosis, which requires two interacting receptors, cubilin and megalin, that form a complex with amnionless [2–4]. Cubilin is a 460-kDa protein encoded by the gene CUBN that contains an N-terminal stretch, 8 epidermal growth factor (EGF)-like repeats, and 27 CUB [complement C1r/C1s, UEGF (EGF-related sea urchin protein) and BMP1 (bone morphogenic protein 1)] domains Figure 1 [5, 6]. Pathogenic variants in the CUBN gene are known to cause Imerslund–Gräsbeck syndrome (IGS) (OMIM #261100), a rare autosomal recessive condition that is characterized by intestinal malabsorption of vitamin B₁₂ resulting in megaloblastic anaemia, frequently accompanied by varying degrees of proteinuria [7, 8]. Most pathogenic CUBN variants causative of IGS are found in the N-terminal half of cubilin, affecting either the interaction with amnionless or the vitamin B₁₂/intrinsic factor-binding CUB domains 5–8 (CUB5–8). Ovunc et al. first described two siblings with isolated proteinuria in the absence of
FIGURE 1: Distribution of CUBN variants along the cubilin protein. Cubilin protein structure summarized with the 8 EGF-like (green) and 27 CUB domains (blue). The orange dots correspond to the theoretical Ca\(^{2+}\) -binding sites. Variants written in blue are associated with IGS in the Human Mutation Database and variants written in black are the previously identified variants causative of isolated proteinuria [9–11]. Variants identified in this study are underlined, and the five novel variants are written in red.

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megaloblastic anaemia carrying CUBN biallelic variants [9]. Later, a second family was reported with isolated proteinuria due to biallelic pathogenic CUBN variants [10]. Recently, C-terminal pathogenic CUBN variants have been reported in patients with isolated proteinuria and normal kidney function; this latter finding contrasts with the general dogma that proteinuria is damaging and eventually causes kidney impairment [11]. Furthermore, genome-wide association studies have identified several C-terminal CUBN variants associated with increased risk of albuminuria [12–16].

In the present study, we report 15 patients from 12 unrelated families bearing biallelic C-terminal variants in the CUBN gene from a cohort of 347 patients presenting proteinuria of suspected monogenic cause. We aimed to perform clinical and genetic characterization of patients with proteinuria caused by anomalies in the CUBN gene.

MATERIALS AND METHODS

Patients

This multicentre study included 347 patients with persistent proteinuria with suspected monogenic cause, referred to our laboratory for genetic testing from several hospitals between January 2014 and April 2020. Signed informed consent was obtained from all patients and/or their parents. The study was approved by Fundació Puigvert Institutional Review Board. Partial data from genetic analysis of 103 patients have been previously published [17].

Genetic testing

Genetic testing was performed in index cases by massive parallel sequencing of a customized capture-based kidney-disease gene panel containing 316 genes, including the CUBN gene, as previously reported [17]. Filtering of the variants was performed using a cut-off minor allele frequency of <0.005 based on population frequency in the Genome Aggregation Database (v3.1) and in our in-house database. Functional impact was predicted by the SnpEff 4.3 program [18]. Missense variants were further evaluated using several pathogenic prediction algorithms included in the dbNSFP v4.0 missense variant database [19]. All identified variants in the CUBN gene were validated by Sanger sequencing and segregation analysis was performed. Variants were classified using American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines [20]. No additional pathogenic sequence variants with relevance for the clinical phenotype of the 15 described patients were identified.

Clinical data

Clinical data from patients with biallelic proteinuria-causing variants in the CUBN gene were retrospectively obtained from medical records, including sex, date of birth, age at diagnosis of proteinuria, urinary protein/creatinine ratio (UProt/Cr), urinary albumin/creatinine ratio, estimated glomerular filtration rate (eGFR) at onset and at last follow-up, treatment received and response, results of kidney biopsy and presence of other renal alterations or extrarenal manifestations. Nephrotic-range proteinuria was defined by a UProt/Cr ratio >2 mg/mg in children and >3.5 mg/mg in adults [21]. eGFR was based on the Schwartz and Chronic Kidney Disease Epidemiology Collaboration equations in paediatric and adult patients, respectively [22, 23], or on creatinine clearance, as available. Baseline characteristics were expressed as frequencies (n, %) and median (range).

RESULTS

Clinical features of patients with CUBN biallelic variants

In a cohort of 347 families with proteinuria of suspected monogenic cause, we identified 15 patients (nine males and six females) from 12 unrelated families with biallelic variants in the CUBN gene, representing 3.5% (12/347) of the cohort. The phenotypic and genetic characteristics of these patients are described in Table 1.

The median age at clinical diagnosis was 4 years (range 0.75–44) with a follow-up duration of 7 years (range 2–39). Three families were reported to be consanguineous, and another three had at least one sibling or cousin with persistent proteinuria. In 80% (12/15) of cases, proteinuria was an incidental finding, and among these, 42% (5/12) occurred during an infection episode. Two patients (P413 and P23-1) had an atypical, more severe phenotype than expected due to C-terminal CUBN variants, likely explained by associated comorbidities.

The remaining 13 patients presented a similar phenotype characterized by chronic isolated non-nephrotic range proteinuria and normal renal function. The median UProt/Cr ratio was 0.83 mg/mg (range 0.50–1.80) at diagnosis and 0.75 mg/mg (range 0.19–2.10) at last follow-up. One patient (P471) presented transient nephrotic-range proteinuria without nephrotic syndrome coincident with an acute febrile illness at diagnosis. Ten patients received treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEi/ARBs) without response. Although not available in all cases, the proportion of albumin in the overall urinary protein was higher than 50%, with a median of 54% (range 42.06–71.71%) at diagnosis and 53.76% (range 39.83–66.68%) at last follow-up. Serum albumin level remained normal and all 13 patients maintained normal renal function over time. Renal biopsy was performed in four patients and no anomalies were identified. None of the 13 patients was hypertensive or presented extrarenal manifestations. The level of vitamin B12 was normal in three patients. Although it was not tested in the rest of patients, none of them presented signs of vitamin B12 deficiency such as megaloblastic anaemia.

Atypical clinical presentation in two CUBN patients

Patient P413 was diagnosed as having steroid-resistant nephrotic syndrome at 3 years of age. His renal biopsy suggested minimal change disease and immunofluorescence was negative. After treatment with corticosteroids and cyclophosphamide, he presented partial clinical and...
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>fam/sp</th>
<th>Age (yr)</th>
<th>UProt/Cr (mg/mg)</th>
<th>UAlb/Cr (mg/g)</th>
<th>eGFR (mL/min/1.73 m²)</th>
<th>Plasma albumin (g/dL)</th>
<th>Treatment, duration (yr)</th>
<th>Renal biopsy (age, yr)</th>
<th>Clinical data at last follow-up</th>
<th>Genetic data</th>
</tr>
</thead>
<tbody>
<tr>
<td>P47-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M</td>
<td>fam</td>
<td>0.8</td>
<td>1.80</td>
<td>836</td>
<td>188.0</td>
<td>4.7</td>
<td>ACEi, 0.2</td>
<td>No</td>
<td>3.0 3.3 2.10 925 156.0 4.9</td>
<td>p.(Tyr3018Ser), h (m) p.(Met3368Val), h (p)</td>
</tr>
<tr>
<td>P47-2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F</td>
<td>fam</td>
<td>4.0</td>
<td>1.27</td>
<td>591</td>
<td>185.0</td>
<td>4.4</td>
<td>ACEi, 0.2</td>
<td>No</td>
<td>3.9 6.8 1.01 482 132.0 4.6</td>
<td>p.(Ser1947Thr), h (p) p.(Gly2691Arg), h (m)</td>
</tr>
<tr>
<td>P507</td>
<td>M</td>
<td>sp</td>
<td>1.3</td>
<td>1.60–2.00</td>
<td>757</td>
<td>153.0</td>
<td>4.0</td>
<td>None</td>
<td>No</td>
<td>2.0 2.7 2.10 1000 180.0 4.3</td>
<td></td>
</tr>
<tr>
<td>P41</td>
<td>M</td>
<td>sp</td>
<td>1.8</td>
<td>1.40</td>
<td>1004</td>
<td>89.6</td>
<td>4.5</td>
<td>ACEI, 11.5</td>
<td>Normal</td>
<td>13.0 15.0 0.95 554 86.5 4.5</td>
<td>p.(Tyr3018Ser), h (m)</td>
</tr>
<tr>
<td>P17</td>
<td>M</td>
<td>sp</td>
<td>2.0</td>
<td>0.85</td>
<td>452</td>
<td>148.5</td>
<td>4.3</td>
<td>ACEI, 4.0</td>
<td>Normal</td>
<td>11.5 13.5 0.59 235 121.5 4.3</td>
<td>p.(Gly2638_Thr2687del), h (p)</td>
</tr>
<tr>
<td>P2</td>
<td>F</td>
<td>sp</td>
<td>2.7</td>
<td>1.57</td>
<td>860</td>
<td>135.7</td>
<td>4.7</td>
<td>ACEI, 2.8</td>
<td>No</td>
<td>3.6 6.6 1.13 588 125.7 4.9</td>
<td>p.(Glu1339Glu), h (m) p.(Asp2786ThrfsTer18), h (de novo)</td>
</tr>
<tr>
<td>P413</td>
<td>M</td>
<td>fam cons</td>
<td>3.0</td>
<td>22.00</td>
<td>15,783</td>
<td>&gt;90.0</td>
<td>1.2</td>
<td>CS/CP/CsA, 7.6</td>
<td>MCD</td>
<td>6.6 10.3 0.96 573 101.0 4.7</td>
<td>p.(Ser1947Thr), h (m)</td>
</tr>
<tr>
<td>P45-1</td>
<td>F</td>
<td>fam</td>
<td>3.0</td>
<td>0.76</td>
<td>N/A</td>
<td>143.0</td>
<td>4.5</td>
<td>None</td>
<td>No</td>
<td>15.7 18.7 0.19 N/A 93.3 4.5</td>
<td>c.7706&gt;1G&gt;T, h (p) p.(Cys3306PhefsTer8), h (m)</td>
</tr>
<tr>
<td>P45-2</td>
<td>M</td>
<td>fam</td>
<td>14.5</td>
<td>0.50</td>
<td>N/A</td>
<td>178.3</td>
<td>4.9</td>
<td>ACEI, 10.5</td>
<td>No</td>
<td>10.5 25.0 0.24 N/A 108.0 4.9</td>
<td>c.7706&gt;1G&gt;T, h (p) p.(Cys3306PhefsTer8), h (m)</td>
</tr>
<tr>
<td>P14</td>
<td>F</td>
<td>sp</td>
<td>5.0</td>
<td>N/A</td>
<td>70%</td>
<td>N/A</td>
<td>4.0</td>
<td>ACEI, 0.5</td>
<td>Normal (27)</td>
<td>39.0 44.8 0.70 468 108.0 4.0</td>
<td>p.(Leu2656_Pro2657delinsPheValValProTyrTer18), h (m)</td>
</tr>
<tr>
<td>P493</td>
<td>M</td>
<td>fam cons</td>
<td>6.0</td>
<td>0.80</td>
<td>490</td>
<td>141.0</td>
<td>4.1</td>
<td>ACEI, 2.0</td>
<td>No</td>
<td>3.0 9.6 0.80 496 124.0 4.7</td>
<td>p.(Ser1947Thr), h (m) p.(Cys1563Ter), h (p)</td>
</tr>
<tr>
<td>P471</td>
<td>M</td>
<td>sp</td>
<td>8.0</td>
<td>&gt;2.00</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>CS/AECI, 0.1/1.0</td>
<td>Normal</td>
<td>7.0 15.1 0.80 N/A 96.1 NA</td>
<td></td>
</tr>
<tr>
<td>P23-1</td>
<td>F</td>
<td>fam cons</td>
<td>35.0</td>
<td>1.91</td>
<td>N/A</td>
<td>103.9</td>
<td>4.3</td>
<td>ACEI/ Allopurinol, NA</td>
<td>FSGS (37)</td>
<td>16.0 51.0 0.95 N/A 70.2 4.3</td>
<td>p.(Tyr3018Ser), h (m) p.(Arg2636Ter), H (m)</td>
</tr>
<tr>
<td>P23-2</td>
<td>F</td>
<td>fam cons</td>
<td>30.0</td>
<td>1.64</td>
<td>N/A</td>
<td>97.6</td>
<td>3.8</td>
<td>ACEI, NA</td>
<td>No</td>
<td>21.6 53.0 1.40 N/A 88.4 3.8</td>
<td>p.(Arg2636Ter), H (m)</td>
</tr>
<tr>
<td>P40</td>
<td>M</td>
<td>fam</td>
<td>44.0</td>
<td>0.54</td>
<td>303</td>
<td>115.0</td>
<td>4.7</td>
<td>ACEI, NA</td>
<td>No</td>
<td>3.0 48.0 0.63 397 126.0 4.7</td>
<td>p.(Tyr3018Ser), h (m)</td>
</tr>
</tbody>
</table>

Cons. consanguineous; CP, cyclophosphamide; CS, corticosteroids; CsA, cyclosporine; A, E, female; fam, familial; FSGS, focal segmental glomerulosclerosis; H, homozygosity; h, heterozygosity; LP, likely pathogenic; M, male; m, maternal; MCD, minimal change disease; N/A, not assessed; p, paternal; sp, sporadic; UAAb/Cr, urine albumin/creatinine ratio; yr, years.

<sup>a</sup>These patients are first cousins.
biochemical remission but moderate proteinuria persisted. Two years later the patient relapsed and again received corticosteroids. His renal function and blood pressure were normal after 6.6 years of follow-up.

Patient P23-1 was diagnosed at 35 years of age with a UP/Prot/Cr ratio of 1.91 mg/mg, focal segmental glomerulosclerosis (FSGS), high blood pressure, hyperuricemia and normal renal function (eGFR 103.9 mL/min/1.73 m²). At last follow-up, at 51 years of age, her renal function had declined (eGFR 70.2 mL/min/1.73 m²), but she presented other comorbidities such as type 2 diabetes mellitus, grade 2 obesity and hypertensive cardiomyopathy. The patient refused to be biopsied during the follow-up time. Remarkably, three out of four of this patient's sisters presented isolated proteinuria and normal renal function, one of whom was also included in this study (P23-2).

Genetic testing by massive parallel sequencing of a 316-kidney disease gene panel did not identify additional pathogenic variants in other genes related to proteinuria in these two patients with a more severe phenotype.

Characterization of C-terminal CUBN proteinuria-causing variants identified

Fourteen different sequence variants were detected in the CUBN gene (Table 2), five of which were novel. The variants p.Ser1947Tyr and p.Tyr3018Ser were found in three families each in this study. Thirteen of the variants were located in the C-terminal half of the protein (CUB10 to CUB25 domains). Only variant p.Glu1339Glu was located in the N-terminal region (CUB8 domain). This variant was found in patient P2 to be inherited from her mother, and was identified together with a C-terminal de novo variant. In the remaining patients, CUBN variants were confirmed to be located in trans.

DISCUSSION

In the current study, we examined a group of 347 families with proteinuria of suspected monogenic cause for the presence of biallelic CUBN variants. We identified 15 patients from 12 unrelated families bearing proteinuria-causing CUBN variants. A total of 14 different variants were detected, of which 13 were located in the C-terminal half of the cubilin protein and five were novel. Patients presented early in life with non-nephrotic isolated proteinuria with normal renal function, in the absence of nephrotic syndrome, hypertension or extrarenal manifestations such as intestinal malabsorption of vitamin B₁₂. Kidney biopsies were normal, and patients did not respond to ACEi/ARB treatment. Characteristically, renal function remained normal over time, without changes in disease phenotype. The proportion of albumin in the overall urinary protein was higher than 50%, very suggestive of a glomerular origin. In clinical practice, albumin predominance in urine helps to exclude a tubulo-interstitial disease as LMW is not measured routinely. That finding supports the differential diagnosis from other rare proteinuric entities characterized by LMW with normal or mildly elevated albumin in urine, such as Dent disease [26] or juvenile cystinosis [27], among others.

Clinical presentation in our cohort resembled that recently reported in patients with biallelic C-terminal CUBN variants [11]. In our study, three patients were above 40 years of age at last follow-up and continued to have chronic non-nephrotic proteinuria and normal renal function. The description of additional adult patients with CUBN variants without kidney impairment adds further evidence that proteinuria due to reduced cubilin function is a benign condition.

Two patients presented a more severe phenotype than would typically be expected in cases of proteinuria due to CUBN variants. Patient P23-1 was a 51-year-old female with type 2 diabetes mellitus, grade 2 obesity and hypertensive heart disease. These comorbidities were more likely the cause of the FSGS and the reduced renal function (eGFR 70.2 mL/min/1.73 m²) than the CUBN variants. The fact that her sister (P23-2 of this cohort) exhibited only isolated proteinuria without progression to chronic kidney disease supports the impact of acquired factors on patient P23-1. Further, the clinical presentation of patient P413 at diagnosis was unique in this cohort. This patient was a 3-year-old boy with nephrotic syndrome (severe proteinuria associated with hypoalbuminemia) at diagnosis, who presented minimal change disease and partial remission with immunosuppression. However, after treatment with corticosteroids and cyclophosphamide his clinical picture was similar to that of the other patients in this cohort, with only isolated moderate proteinuria, and renal function was preserved in the absence of any subsequent treatment. We speculate that in this patient, idiopathic nephrotic syndrome of benign course superimposed on his genetic condition, leading to the need for more aggressive management [28]. Patient P413 harboured the missense variant p.Ser1947Tyr in homozygous state. Interestingly, this variant was also carried in the homozygous state by another patient (P493) with the characteristic phenotype described for patients with biallelic C-terminal CUBN variants.

Genotype–phenotype correlations in patients carrying biallelic CUBN variants have been established with respect to variant localization (Figure 1) [4, 29]. Most patients with CUBN pathogenic variants located in the N-terminal half of the protein (before the CUB8 domain) were found to be affected by IGS. The disorder results from a combination of vitamin B₁₂ deficiency due to selective malabsorption of the vitamin, and impaired reabsorption of LMW proteins in the proximal renal tubule. Life-long treatment with vitamin B₁₂ results in sustained clinical improvement of the anaemia and resolution of the neurologic symptoms, if present. Mild persistent proteinuria with normal kidney function is present in about half of the patients. In contrast, variants located in the C-terminal half after the vitamin B₁₂/intrinsic factor-binding domain were detected in patients with isolated proteinuria with a high proportion of albuminuria, and without impairment of kidney function [9–11]. Fourteen of the patients presented in this study carried both variants after CUB8. The remaining one (P2) carried one variant within the CUB8 domain likely in trans with another variant located after CUB8. Similarly, two patients described by Bedin et al. carried one variant before CUB8 in trans with one C-terminal CUBN variant, also with presentation of isolated proteinuria and normal kidney
### Table 2. Classification of the variants identified in the CUBN gene in patients with proteinuria.

<table>
<thead>
<tr>
<th>CUBN variant (NM_001081.4)</th>
<th>Exon</th>
<th>Protein domain</th>
<th>Minor allele frequency (gnomAD)</th>
<th>In silico algorithm predictions</th>
<th>Reference</th>
<th>ACMG criteria</th>
<th>Patients (zygosity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.4017G&gt;A p.(Glu1339Glu)/p.(?)</td>
<td>27</td>
<td>CUB8</td>
<td>Absent</td>
<td>D (ADA: 1.00) D (RF: 0.75)</td>
<td>Novel</td>
<td>LP (PM1, PM2, PM3, PP3)</td>
<td>P2h</td>
</tr>
<tr>
<td>c.4689_4690delinsAT</td>
<td>31</td>
<td>CUB10</td>
<td></td>
<td>–</td>
<td>(10)</td>
<td>P (PVSI, PM1, PM2)</td>
<td>P471h</td>
</tr>
<tr>
<td>c.5840C&gt;A p.(Ser1947Tyr)</td>
<td>39</td>
<td>CUB13</td>
<td>0.0002 (0.02%)</td>
<td>TDDDDTTDDTTDD</td>
<td>[24]*</td>
<td>VUSb (PM1, PM2, PM3_supportingc)</td>
<td>P507 hp493 HP413 H</td>
</tr>
<tr>
<td>c.7706–1G&gt;T p.(?)</td>
<td>49</td>
<td>IVS 49</td>
<td>Absent</td>
<td>–</td>
<td>(11)</td>
<td>P (PVSI, PM1, PM2, PM3)</td>
<td>P45-1 h P45-2 h</td>
</tr>
<tr>
<td>c.7906C&gt;T p.(Arg2636Ter)</td>
<td>50</td>
<td>CUB19</td>
<td>0.0001 (0.01%)</td>
<td>–</td>
<td>[25]</td>
<td>P (PVSI, PM1, PM2, PM3)</td>
<td>P23-1 H P23-2 H</td>
</tr>
<tr>
<td>c.7913–3_8062+3del</td>
<td>51</td>
<td>CUB19</td>
<td>Absent</td>
<td>–</td>
<td>Novel</td>
<td>LP (PM1, PM2, PM4)</td>
<td>P17h</td>
</tr>
<tr>
<td>c.7968_7969delIGCins</td>
<td>51</td>
<td>CUB19</td>
<td></td>
<td>–</td>
<td>(11)</td>
<td>LP (PM1, PM2, PM3)</td>
<td>P14 H</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>c.8017G&gt;A p.(Gly2691Arg)</td>
<td>52</td>
<td>CUB20</td>
<td>0.0003 (0.03%)</td>
<td>TDDDDDDTDDTD</td>
<td>[7]</td>
<td>VUSb (PM1, PM2)</td>
<td>P507 h</td>
</tr>
<tr>
<td>c.8355del p.(Asp2786ThrfsTer18)</td>
<td>53</td>
<td>CUB20</td>
<td>0.000004 (0.0004%)</td>
<td>–</td>
<td>(9)</td>
<td>P (PVSI, PM1, PM2)</td>
<td>P2h</td>
</tr>
<tr>
<td>c.9053A&gt;C p.(Tyr3018Ser)</td>
<td>57</td>
<td>CUB22</td>
<td>0.0001 (0.01%)</td>
<td>TTTDDTTDDDDTD</td>
<td>(11)</td>
<td>LP (PM1, PM2, PM3)</td>
<td>P471 hp40 H P47-1 h P47-2 H</td>
</tr>
<tr>
<td>c.9555T&gt;G p.(Cys3185Trp)</td>
<td>60</td>
<td>CUB24</td>
<td>0.00044 (0.004%)</td>
<td>TDDDDTTDDDDTD</td>
<td>Novel</td>
<td>LP (PM1, PM2, PM3)</td>
<td>P45-1 h P45-2</td>
</tr>
<tr>
<td>c.9910_9916dup p.(Cys3306PhefsTer8)</td>
<td>62</td>
<td>CUB25</td>
<td>0.000004 (0.0004%)</td>
<td>–</td>
<td>Novel</td>
<td>LP (PM1, PM2, PM3)</td>
<td>P41 H</td>
</tr>
<tr>
<td>c.9922T&gt;C p.(Trp3038Arg)</td>
<td>62</td>
<td>CUB25</td>
<td>0.0001 (0.01%)</td>
<td>TDDDDDDTDDTD</td>
<td>[11]</td>
<td>LP (PM1, PM2, PM3_supportingc, PP3) VUSb (PM1, PM2, PM3, BP4)</td>
<td></td>
</tr>
<tr>
<td>c.10102A&gt;G p.(Met3368Val)</td>
<td>63</td>
<td>CUB25</td>
<td>0.0002 (0.02%)</td>
<td>TTTTTTTTTTTTT</td>
<td>Novel</td>
<td></td>
<td>P47-1 h</td>
</tr>
</tbody>
</table>

D, damaging; gnomAD, Genome Aggregation Database; H, homozygosity; h, heterozygosity; LP, likely pathogenic; P, pathogenic; T, tolerated; VUS, variant of uncertain significance.

*Patient P413 already reported.

bThese variants were considered proteiniuria causing in this manuscript even though they were strictly VUS according to the ACMG/AMP guidelines.

cReduced one evidence level due to homozygous occurrence.

dVariant located at the last nucleotide of CUBN exon 27.

The order of the predictions for the missense pathogenicity predictors (dbNSFP v4.0a) is as follows: BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, IJST-S2, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI and SIFT. Splicing pathogenicity predictors (dbscSNV v1.1): ADA and RE.

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function [11]. Although the level of vitamin B12 was not tested in all patients, none of them presented signs of vitamin B12 deficiency. Thus, systematic supplementation of vitamin B12 is probably not useful in patients with biallelic C-terminal variants. Patients with truncating or missense variants showed no phenotypic differences. Most of the proteinuria-causing variants identified so far in CUBN are unique to single families (‘private variants’). The most prevalent causative CUBN variant identified in families with isolated proteinuria and normal kidney function is p.Tyr3018Ser, which has been reported in a total of 10 families: seven previously reported families [11] plus three families from this study.

The 2015 ACMG/AMP guidelines established a classification system for sequence variants that has allowed harmonization of variant interpretation across laboratories and countries [20]. However, the broad scope of these guidelines necessitates specification of evidence types for specific genes or diseases of interest [30–34]. The ACMG/AMP guidelines proposed strict use of the term ‘likely pathogenic’, reserving it for variants with greater than a 90% certainty of being disease causing. This narrow interval of pathogenicity is important for severe monogenic diseases for which identification of pathogenic and likely pathogenic variants may have therapeutic or reproductive consequences, but may not be applicable to variants causative of more benign conditions. Biallelic C-terminal CUBN variants cause chronic proteinuria with normal renal function, which is a benign condition that has no effect on reproductive fitness. Thus, the allele frequency threshold for variants that cause this benign condition can be higher than that established for severe recessive diseases [33, 34].

Applying the ACMG/AMP classification criteria, three of the identified variants were classified as variants of uncertain significance (VUS) (Table 2). However, we considered these variants causative of chronic proteinuria with normal renal function for several reasons: (i) they were identified in trans with another rare CUBN variant or in homozygous state; (ii) the phenotype of patients carrying these variants fitted with the CUBN gene; and (iii) two of these variants were present in multiple patients in this cohort. This work adds evidence that C-terminal CUBN variants cause chronic proteinuria with normal renal function. In addition, the characteristics of the CUBN variants identified would help to define CUBN-specific rules for variant classification, which could also be applied to other genes with a recessive mode of inheritance and causative of benign conditions with onset at paediatric age. Further studies of patients with biallelic C-terminal CUBN variants and functional studies, together with expert specifications of the ACMG/AMP variant interpretation guidelines for benign recessive conditions, will shed more light on the classification and causality of CUBN variants identified in the future.

Genetic studies performed in adult cohorts of patients with chronic kidney disease have not identified biallelic C-terminal variants in the CUBN gene [35–37]. Neither a genetic study of a cohort of patients who received a kidney transplant before 25 years of age [38] nor one of a cohort of kidney transplant recipients aged <50 years [39] identified pathogenic variants in the CUBN gene. However, other genetic studies have reported a few paediatric patients with biallelic CUBN variants. In a cohort of 362 families presenting with proteinuria and haematuria before the age of 25 years, three patients from the same family were reported to bear a homozygous pathogenic variant in the CUBN gene. The three siblings, with ages at diagnosis of 12 years, 5 years and 1 month, presented non-nephrotic range proteinuria and microhaematuria [40]. A Japanese cohort of 230 patients with severe proteinuria found only one patient with biallelic CUBN variants. This patient was a 3-year-old child with a kidney biopsy showing minor glomerular abnormalities and normal kidney function [41]. In a cohort of 1783 families with steroid-resistant nephrotic syndrome that manifested before 25 years of age, five families were reported to carry biallelic variants in the CUBN gene [9, 42]. One family with FSGS harboured the homozygous CUBN variant p.Gly1840Ser, which later was observed with high allelic frequency in the Genome Aggregation Database (7.571%, with 2220 homozygous) and classified as benign in ClinVar (VCV000299459.4, accessed 19 May 2021). In another family, two siblings presented with intermittent nephrotic-range proteinuria [9], in agreement with the isolated proteinuria cases described here and in other studies [10, 11]. All these studies are in line with our finding that proteinuria caused by C-terminal CUBN variants is mainly detected in young patients, mostly with isolated non-nephrotic proteinuria without progression to chronic kidney disease and lack of response to ACEI/ARB treatment.

Better understanding of the potential genetic causes of proteinuria should encourage genetic testing in patients with chronic proteinuria, especially in young patients with a family history of proteinuria and/or consanguinity. Genetic testing enables the aetiologic diagnosis of rare genetic causes of proteinuria in children and young adults, such as Dent disease or juvenile cystinosis, which are commonly overlooked [25, 43, 44], as well as of glomerulopathies related to LMX1B and PAX2, for which clinical presentation may overlap [45, 46]. Taken together, all these findings and considerations support the value of genetic testing using massive parallel sequencing in order to achieve an accurate aetiologic diagnosis of proteinuria and improve patient management.

In conclusion, this study confirms that biallelic C-terminal variants in the CUBN gene cause a benign proteinuric condition characterized by isolated chronic proteinuria and normal kidney function. This study should help to increase awareness among nephrologists of the good prognosis of these patients and to avoid unnecessary kidney biopsies and inefficient treatment for reduction of glomerular proteinuria.

ACKNOWLEDGEMENTS

We acknowledge the patients for taking part in this study and the referring physicians who participated. We thank Patricia Ruiz and Laura Lorente from Fundació Puigvert for technical support for genetic testing. We also thank the Catalan Government (AGAUR 2017/SGR-00676) and IIB Sant Pau Biobank for providing some of the samples.
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AUTHORS’ CONTRIBUTIONS


FUNDING

This project was funded by the Instituto de Salud Carlos III (ISCIII)/FEDER funds: PI18/00362, PI19/01633, RETIC RED- INREN RD16/0009/0019 and Plataforma ISCIII Biobancos PT20/00196.

CONFLICT OF INTEREST STATEMENT

None declared.

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Received: 23.6.2021; Editorial decision: 25.8.2021