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Genetic testing, a challenge to kidney biopsy? A case report

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Abstract

The field of genetic testing has experienced significant growth in medical practice since 1956, when the first genetic analysis was introduced. Persistent proteinuria has long been considered a strong risk factor for the progression of chronic renal failure, though, paradoxically, it can also be a benign process, as seen in individuals with mutations in the cubilin (CUBN) protein, specifically the C-terminal. CUBN is a peripheral protein that plays a crucial role in the receptor-mediated endocytotic reabsorption of albumin in the proximal tubule. In the past, there have been misinterpretations of CUBN variants with isolated proteinuria as glomerular injury, leading to unnecessary kidney biopsies and ineffective treatments. This paper discusses two siblings with a homozygous variant of (p.Tyr3018Ser) in the C-terminal of the CUBN protein, inherited from both heterozygous carrier parents. This case presents an opportunity to question our typical approach to proteinuria in an effort to avoid unnecessary kidney biopsies and the subsequent side effects of treatments, particularly for those with proteinuria.

Keywords: cubilin, proteinuria, albuminuria, p.Tyr3018Ser, genetic testing, kidney biopsy, children

Introduction

Genetic analysis in the field of cytogenetics began when Tjio and Levan reported the correct number of human chromosomes in 1956. From this point onwards, genetic testing has grown at a rapid pace in medical practice. Currently, genetic testing is available for over 2000 genetic conditions, including kidney diseases, at affordable prices [1-3].

Persistent proteinuria, especially albuminuria, is known to be a significant risk factor for the progression of chronic kidney disease (CKD) [4]. The main cause of proteinuria is a defect in the glomerular filtration barrier, possibly combined with dysfunction in proximal tubular protein reabsorption. As such, kidney biopsies have long been recommended, primarily to exclude glomerular diseases in children with persistent proteinuria before initiating a proteinuria-lowering treatment [5].

However, recent publications suggest that not all forms of proteinuria may be destructive, particularly in cases exhibiting albuminuria due to the diminished function of cubilin (CUBN) [5-7]. Follow-up studies on such cases show that isolated proteinuria, caused by mutations in the CUBN gene, is benign and does not negatively impact the long-term prognosis of kidney function [5-7].

CUBN, encoded by the CUBN gene, is a 460 kDa peripheral protein that forms the uptake receptor complex in the proximal tubule. This complex is constituted of transmembrane proteins, megalin, and amnionless, with an N-terminal of 110 amino acids, eight EGF-like domains, and 27 CUB domains [5]. A limited amount of albumin, filtered from the glomerulus, is physiologically reabsorbed via receptor-mediated endocytosis, a process dependent on CUBN in the proximal tubule [5,6].

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Informed Consent
The authors stated that the written consent was obtained from the parents of the patient presented with images in the study.

Conflict of Interest
No conflict of interest was declared by the authors.

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Uncovering genetic risk factors through prompt genetic analysis may encourage clinicians to revise traditional strategies used for diagnosing and treating underlying disorders of proteinuria. Could genetic analysis be a viable challenge in questioning the need for a kidney biopsy and the initiation of empirical immune-suppressive treatment in chronic proteinuria?

In this context, we present two siblings with a homozygous variant (p.Tyr3018Ser) on the 22nd domain of the CUBN's C-terminal. These siblings come from a nuclear family in which both parents are heterozygous carriers, although there is no consanguinity. The aim is to instigate a debate about the classical approach to proteinuria.

Case presentation

Admittedly, we obtained written parental consent in advance to share the medical history of her sons in a medical article or any medical discussion context.

Initial presentation

Patient A, a boy who recently turned 13, was consulted for incidentally detected 2+ proteinuria during a urine analysis. There was neither a family history of proteinuria nor any kidney disease, nor was there any consanguinity between his parents. Upon the initial check, no clinical manifestations were observed. Additionally, both ophthalmologic and hearing examinations showed no significant abnormalities.

Laboratory results

Kidney function was normal, with a serum creatinine of 0.55 mg/dl. Plasma albumin level was 4.6 g/dl. Urinary protein was 0.54 g/24 h. Complete blood count (CBC), immunologic data (Complement 3 (C3), 1.77 g/L (N: 0.86–1.82 g/L) and complement 4 (C4), 0.35 g/L (N: 0.17–0.51 g/L), ANA (-), anti-dsDNA-10.33 IU/ml (N:<100 IU/ml)). However, CRP was 3.4 mg/L (N: 0.0–5.0 mg/L). Urine protein electrophoresis revealed that albumin-64.38% (N: 55.8–66.1%), α1-%4.27 (N: 2.9–4.9%), α2-8.95% (N: 7.1–11.8%), β1-5.92% (N: 4.7–7.2%), β2- 3.43% (N: 3.2–6.5%),γ-13.04% (N: 11.1–18.8%). A urinary ultrasound examination showed normal kidneys and a normal urinary system.

Clinical course

In the subsequent 3 months, urinary protein excretions varied between 0.5 and 4 g/day (Table 1). A renal biopsy was performed when the patient presented with severe proteinuria over the nephrotic range (>4 g/day) and mild hypoalbuminemia, exhibiting a serum albumin of 3.1 g/dl, in addition to mild to moderate general edema. The pathological examination of the kidney specimen disclosed minor changes in glomeruli with prominent podocytes, with no immunological staining observed. Electron microscopy demonstrated minimal irregularities in the basal membrane and a slight increase in the mesangial matrix; the epithelium and capillary walls were intact (Figure 1). Given the severe proteinuria, the possibility of Minimal Change Disease was excluded. Consequently, immune-suppressive treatment with prednisone and the renin-angiotensin-aldosterone system blocker Ramipril was initiated while waiting for the biopsy results. Over a 10-week period, there was no response to the treatment. The clinical presentation indicated a steroid-resistant process, which was bolstered by the recent discovery of ++ proteinuria without hypoalbuminemia in the patient's younger sibling. The immune-suppressive treatment was then discontinued, while Ramipril was continued in consideration of a potentially genetically transmitted disorder. The elevated level of proteinuria was expected to decrease spontaneously over time. Currently, the boy is 16 years old and has lived for 3 years post-diagnosis. The most recent tests showed urine protein, creatinine, and serum albumin levels of 0.35 g/day, 0.47 mg/dl, and 4.1 g/dl, respectively.

Patient B, the younger brother, was first diagnosed with 2+ proteinuria at the age of 5 during a family examination. Despite the diagnosis, he had no complaints or significant abnormalities in his blood biochemical data or clinical findings. A kidney biopsy was not considered necessary. Thus, no treatment was initiated. At the time of writing this report, his latest serum albumin level was noted to be 4.4 g/dl, with his serum creatinine at 0.39 mg/dl and urine protein at 0.15 g/d.

Following the detection of proteinuria (++ level) in a younger boy during family screening, genetic testing was conducted for both siblings and their parents. No pathologic variant was identified in the COL4A3, COL4A4, or COL4A5 genes using Next Generation Sequencing (NGS) to analyze for Alport syndrome in the boys. However, the homozygous variant "NM_001081.4(CUBN):c.9053A>C (p.Tyr3018Ser)" was found upon performing Sanger sequencing in both patients, with each parent exhibiting a heterozygous variant.

Figure 1: Minor irregularities in basal membrane and a mild increase in mesangial matrix with intact epithelium and capillary walls.

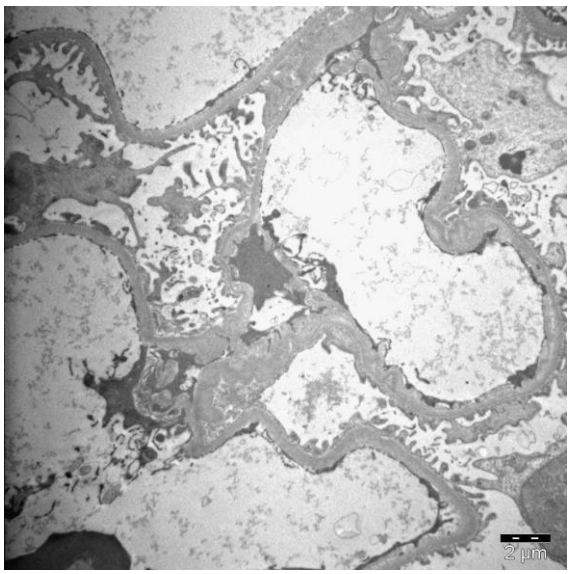

Table 1: Summary of the chronologic clinical record of the family.

	Patient A	Patient B	Mother	Father
1st admission-3months	(++) proteinuria Upr=0.5-4 g/d N serum Alb N RF	(+/++) proteinuria N serum Alb N RF	Clinically N No proteinuria	Clinically N No proteinuria
3months-1st year	(++++) proteinuria Upr=4g/d Serum Alb=3.1g/dl N RF Biopsy (minor changes)	(+/++) proteinuria Nserum Alb N RF	Clinically N No proteinuria	Clinically N No proteinuria
1st-2nd year	(+/++) proteinuria N serum Alb N RF	(+/++) proteinuria N serum Alb N RF	Clinically N No proteinuria	Clinically N No proteinuria
2nd-3rd year	(+/++) proteinuria N serum Alb N RF	(+/++) proteinuria N serum Alb NRF	Clinically N No proteinuria	Clinically N No proteinuria
3rd year-	(+/++) proteinuria N serum Alb N RF	(+/++) proteinuria N serum Alb N RF	Clinically N No proteinuria	Clinically N No proteinuria

Upr: Urine Protein, N: Normal, Alb: Albumin, RF: Renal function

Discussion

Proteinuria has long been recognized as a clinical sign of kidney injury, which can alert clinicians to a potential risk of progression to chronic kidney disease. However, there is limited information on whether all forms of proteinuria are harmful to patients. The primary cause of proteinuria involves disorders of the glomerular filtration barrier, in conjunction with a newfound possibility for harmless effects stemming from defects in proximal tubular protein reabsorption [5,8].

Plasma proteins, chiefly albumin, undergo size-selective glomerular filtration, followed by tubular reabsorption in the proximal tubule through receptor-mediated endocytosis, allowing normal urine to be protein-free. The concentration of albumin in the glomerular ultrafiltrate is reported to be 1 to 50 g/ml, and it is reabsorbed in the initial proximal tubule regardless of the amount under physiological conditions [8]. The uptake receptor complex comprises transmembrane proteins, namely megalin (LRP2) and amnionless (AMN), and a peripheral protein, CUBN, which could be the main albumin receptor in the proximal tubules [5,9,10]. Each CUBN protomer has 8 EGF domains and 27 CUB (complement C1r/C1s, UEGF [EGF-related sea urchin protein] and bone morphogenic protein 1) domains [5]. Unlike mutations mostly in the N-terminal and those in the Vitamin B12/intrinsic factor-binding (IF-binding) CUB domains 5–8, which cause Imerslund-Gräsbeck syndrome (IGS), the variants after the Vitamin B12/intrinsic factor-binding domain result in albuminuria, highlighting that C-terminal CUB domains are crucial for tubular protein reabsorption, without impairing renal filtration function [5,10]. The cases presented here possessed a homozygous p.Tyr3018Ser mutation on the 22nd domain, which is located in the C-terminal, similar to previous cases with isolated proteinuria due to CUBN variants in published cohorts [5–7,11].

Yet, there have been reports claiming that proteinuria was not associated with an unfavorable prognosis, thus preventing chronic kidney disease [5,6,9]. Bedin et al. [5] revealed that high urinary albumin was often misinterpreted as glomerular injury in patients in their cohort, leading to unnecessary kidney biopsies and subsequent protein-lowering treatments despite the presence of clinically benign CUBN variants. Accordingly, they argued that detecting CUBN variants could help avoid inefficient therapies and invasive procedures, particularly when diagnosing cases with subnephrotic proteinuria [5]. We concur with Bedin's claim, considering the favorable prognosis observed in the two siblings with minor changes in kidney biopsy presented here after a three-year follow-up. Likewise, we recommend that genetic testing be considered well before a biopsy in children with persistent proteinuria to avoid both unnecessary procedures and ineffective treatments.

Since Ovunc et al. [7] first reported two patients with proteinuria due to mutations in the CUBN genes, over 60 cases have been reported worldwide, with c.9053A>C being the most frequently mutated locus, as in the family presented here. Diagnoses of CUBN-mediated proteinuria typically occur in childhood, with an average age of 4 to 10.9 years at clinical diagnosis in different cohorts [5,11]. The male-female ratio was 3:2 [6]. Consistent with previous data, the patients presented herein were both males, ages 13 and 5.

The CUBN domains provide ligand binding sites for various proteins, particularly for the intrinsic factor-vitamin B12 complex and albumin, thereby facilitating their intestinal and renal absorption. The albumin binding site is exclusively the C-terminal structural domain, located subsequent to CUBN domains 5-8, where the intrinsic factor-vitamin B12 complexes bind [5,6]. In the cases presented herein, the mutation occurs in the C-terminal, with no corresponding vitamin B12 deficiency or loss of renal function, consistent with findings already documented in the literature.

While proteinuria due to mutations in CUBN genes is usually subnephrotic, Ovunc et al. [7] reported two cases with intermittent nephrotic-range proteinuria. In cohorts where albuminuria is caused by C-terminal variants in the *CUBN* gene, kidney function has been reported to be preserved for over 7 years or until adulthood [5,11]. A common thread in diverse cohorts with persistent proteinuria due to CUBN mutations has been a benign course with normal kidney biopsy results and preserved kidney function over time despite a lack of response to treatment. In the siblings presented here, renal function has been individually maintained for over 3 years despite ongoing mild proteinuria and no response to Angiotensin-converting enzyme inhibitors (ACE) inhibitors and steroids.

The use of genetic testing has become more common recently, especially in patients with unexplained proteinuria, revealing more information about etiology and prognosis prediction [5,11]. Sanger sequencing is used to classify variants in the CUBN gene, following guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [12]. In cohorts of proteinuria secondary to CUBN gene mutations, the diagnostic power of a renal biopsy was negligible compared to genetic testing. In these cases, the results were either normal or showed minimal lesions, as was the case presented here [5,7,11].

In this regard, I argue that not all forms of isolated proteinuria are originally damaging to the glomeruli but might be benign, similar to cases with CUBN variants. Hence, the initial detection of CUBN variants through genetic testing can prevent unnecessary renal punctures and ineffective therapies, which may bring side effects besides leading to a diagnosis. I strongly propose that the typical approach of nephrologists to isolate proteinuria needs to be reevaluated for potential updates. Considering genetic testing as an initial step before contemplating a renal biopsy or starting treatment in patients with isolated subnephrotic proteinuria could be a more judicious approach unless there are atypical clinical scenarios such as nephrotic syndrome or deteriorating kidney function.

Patient perspective

The family has always been cooperative and eager to receive a proper diagnosis. They have formed a positive relationship with the clinician in charge of their care throughout the follow-up period.

Conclusion

In conclusion, isolated persistent proteinuria may not always indicate a glomerular disorder with a high risk for a poor prognosis, which would justify a kidney biopsy. Instead, it could potentially have a benign nature. Genetic testing, such as Sanger sequencing, challenges the traditional approach to persistent

proteinuria. It argues for its prioritized use before considering a biopsy because it promises to prevent unnecessary procedures and pointless treatments that could have potential side effects.

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