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The Bench-to-Bedside Transition

Gitelman syndrome and glomerular proteinuria: a link between loss of sodium-chloride cotransporter and podocyte dysfunction?

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ABSTRACT

We report on a 27-year-old patient presenting with chronic hypokalaemia, inappropriate kaliuresis, hypomagnesaemia and alkalosis, associated with moderate proteinuria. Genetic analysis evidenced a homozygous mutation (p.Arg399Cys) in the *SLC12A3* gene coding for the sodium-chloride cotransporter (NCC), confirming the diagnosis of Gitelman syndrome. Further genetic testing did not show any mutation in *NPHS2*. A renal biopsy was performed in view of the unusual association with proteinuria. Light microscopy showed hypertrophy of the juxtaglomerular apparatus and discrete mesangial thickening. In addition to possible focal segmental glomerular sclerosis lesions, electron microscopy showed extensive segments of variably thickened glomerular basement membrane (GBM), contrasting with segments of regular GBM of low range thickness, and effacement of podocyte foot processes. Of interest, alterations of the GBM were also observed in a *Slc12a3* knock-out mouse model for Gitelman syndrome. These data suggest that the association between Gitelman syndrome and secondary changes of the GBM is probably not coincidental. Possible mechanisms include angiotensin II- or renin-induced podocyte lesions, as well as chronic hypokalaemia.

Keywords: glomerular basement membrane, hypokalaemia, secondary aldosteronism, tubulopathy

INTRODUCTION

Gitelman syndrome is a recessively inherited salt-losing tubulopathy characterized by hypokalaemia, hypomagnesaemia, inappropriate kaliuresis and hypocalciuria. The majority of cases are caused by loss of function mutations in the *SLC12A3* gene coding for the thiazide-sensitive sodium-chloride cotransporter (NCC), normally expressed in the apical membrane of the renal distal convoluted tubule [1]. Gitelman syndrome is considered as the most frequent inherited tubulopathy, with a prevalence of heterozygotes at ∼1% in European populations [2]. Although Gitelman syndrome is a disease of the distal tubule, there have been few reported cases associated with glomerular lesions including focal segmental glomerulosclerosis (FSGS) [3] and C1q nephropathy [4]. To date, no association of Gitelman syndrome with abnormal findings of the glomerular basement membrane (GBM) has been reported.

CASE REPORT

A 27-year-old Caucasian man was referred for evaluation of hypokalaemia, hypomagnesaemia and moderate proteinuria. His past medical history included tetany at the age of 16 years, which led to the discovery of hypokalaemia and hypomagnesaemia. The patient complained of salt craving, polydypsia

and occasional nocturia. He had frequent cramps in the calves and paresthesias of the face and hands. Physical examination revealed a sitting blood pressure of 100/75 mmHg. Laboratory tests confirmed hypokalaemia (2.5 mmol/L), hypomagnesaemia (1.17 mmol/L) and alkalosis (plasma bicarbonate 33 mmol/L). Kidney function was normal (plasma creatinine 0.95 mg/dL, eGFR 95 mL/min/1.73 m^2). Urinalysis revealed inappropriate kaliuresis (104 mmol/24 h), abundant natriuresis (266 mmol/24 h), hypocalciuria (<20 mg/24 h) and proteinuria of 770 mg/24 h. Proteinuria, mostly albuminuria, was confirmed at 1200 mg/24 h in a second sample. Beta-2 microglobulinuria was normal (<0.2 mg/L) and the urinary sediment was bland.

Sequence analysis of the *SLC12A3* gene revealed a homozygous mutation in exon 10 (c.1195C>T). This change, which is predicted to substitute the arginine at codon 399 by a cysteine residue (p.Arg399Cys, R399C), is considered to be a recurrent-disease causing mutation (variation: CM014400) [5]. The mutation was found at the heterozygous state in the father and at the homozygous state in the sister of the patient. None of them had proteinuria. There was no mention of consanguinity in this family. Of note, the parents of the proband originate from two villages located in the eastern part of Belgium where the R399C variant has been detected previously in unrelated cases.

The unusual association of Gitelman syndrome and proteinuria in the proband prompted us to perform a renal biopsy (Figure 1). Light microscopy showed hypertrophy of the juxtaglomerular apparatus and discrete mesangial thickening without hypercellularity. There was no interstitial fibrosis or tubular atrophy. No immune deposits were detected by immunofluorescence. Two small glomerular tufts limited by a thickened Bowman's capsule containing patent capillary loops with a slightly increased thickness of their wall were available in the sample for electron microscopic examination. In some areas, the observation of lipoprotein droplets in the cytoplasm of podocytes occasionally detached from smooth regular but collapsed glomerular basement membranes is consistent with the development of FSGS lesions. In addition, there were extensive segments of variably thickened GBM with clarifications and densities, contrasting with segments of smooth regular GBM. All these altered GBMs displayed quite extensive effacement of foot processes but preservation of the slit diaphragms (Figure 1). The glomerular lesions motivated a further sequence analysis of the exons and flanking intronic regions of the *NPHS2* gene to exclude the presence of the R229Q variant, which is present in 3.6% of controls and considered as a susceptibility factor for late-onset FSGS [6]. This analysis did not show any aberration in the *NPHS2* sequence of the proband.

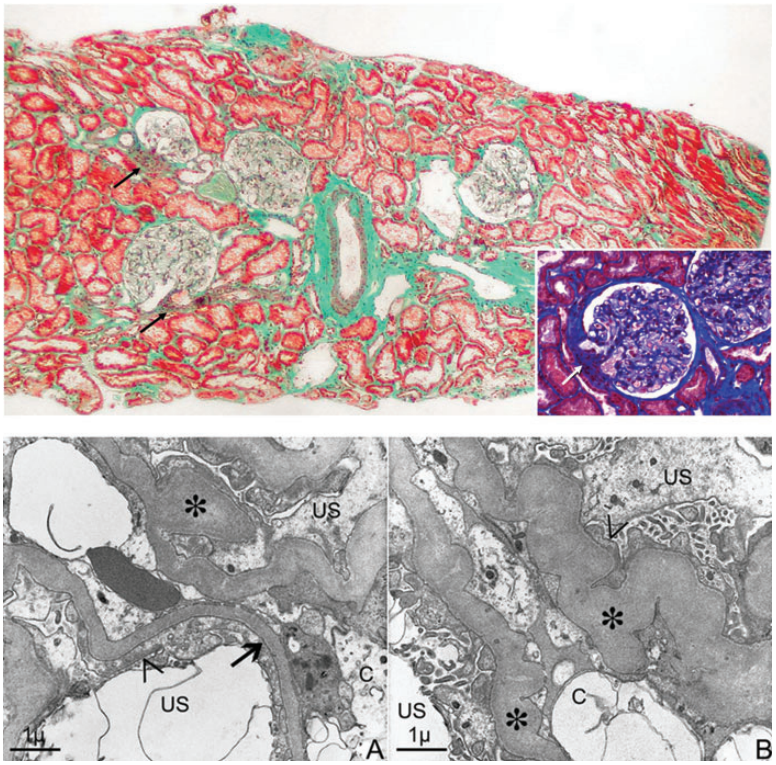

FIGURE 1: Renal biopsy of the index case presenting with Gitelman syndrome and glomerular proteinuria. Upper panel: (Masson's trichrome green, obj. ×4, and blue (insert), obj. ×20). Light microscopic examination showed hypertrophy of the juxtaglomerular apparatus (arrows) and discrete mesangial thickening without hypercellularity. There were no interstitial fibrosis and no tubular atrophy or additional parenchymal alterations. No immune deposits were detected by immunofluorescence. Lower panels: (Epon, lead stain) Electron microscopy revealed significant alterations of the glomerular basement membrane (GBM), with extensive segments presenting severe irregular contours varying in thickness containing clarifications and densities (asterisk) or with smooth regular contours of GBM at the lower range of thickness (mean: 306 nm) (arrow). All altered GBMs display quite extensive effacement of foot processes (arrowhead). C, glomerular capillary lumen; US, urinary space.

In order to substantiate the potential association of glomerular defects with Gitelman syndrome, we analysed kidneys from *Slc12a3* knock-out mice and their littermate controls [7]. Although the glomeruli of NCC-deficient mice appeared normal at the light microscopy level, electron microscopy showed several circumscribed GBM protrusion. These irregular GBM thicknesses were accompanied by focal foot process effacements and the occasional formation of pseudocysts in podocytes (Figure 2). These changes, not observed in the kidneys of control mice, were similar to those seen in the proband.

DISCUSSION

To our knowledge, we report here the first case of genetically proven Gitelman syndrome associated with glomerular proteinuria and significant abnormalities of the GBM. Defects of the GBM and podocytes were also detected in the *Slc12a3* knock-out mouse model. The lesions observed in the index case and the mouse model points to a possible link between the functional loss of NCC and podocyte dysfunction. The R399C mutation detected in the homozygous state in the proband is known to be particularly severe. Functional studies in *Xenopus* oocytes showed that R399C mutants are not complex glycosylated, absent from the plasma membrane, and non-functional, and they have been identified in a subset of severely affected males [5].

Salt-losing tubulopathies are not classically associated with proteinuria, and the few renal biopsies available most often show hyperplasia of the juxtaglomerular apparatus, with no glomerular and no tubular abnormalities [8]. However, a retrospective analysis of patients with genetically proven Gitelman syndrome revealed proteinuria with preserved renal function in 6/36 patients. Three of these six patients were hypertensive; no renal biopsy was performed [9]. Isolated cases of FSGS [3] and C1q nephropathy [4] have also been reported in association with Gitelman syndrome. Long-term follow-up of patients with classic Bartter syndrome secondary to biallelic mutations in *CLCNKB* showed mild to moderate glomerular proteinuria in 6/13 patients, with associated microhaematuria in two of the six patients. Renal biopsy performed in two patients revealed mesangial expansion/hypertrophy without juxtaglomerular apparatus hypertrophy [10]. No ultrastructural examination of the glomeruli was reported.

What could account for the association of Gitelman syndrome with podocyte defects and subsequent alteration of the GBM and proteinuria?

One hypothesis is that the chronic activation of the renin-angiotensin-aldosterone pathway, leading to increased systemic and local levels of angiotensin-II and renin, may in turn cause podocyte lesions. Angiotensin-II can induce proteinuria through haemodynamic and non-haemodynamic mechanisms involving the vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 [11]. Of note, GBM

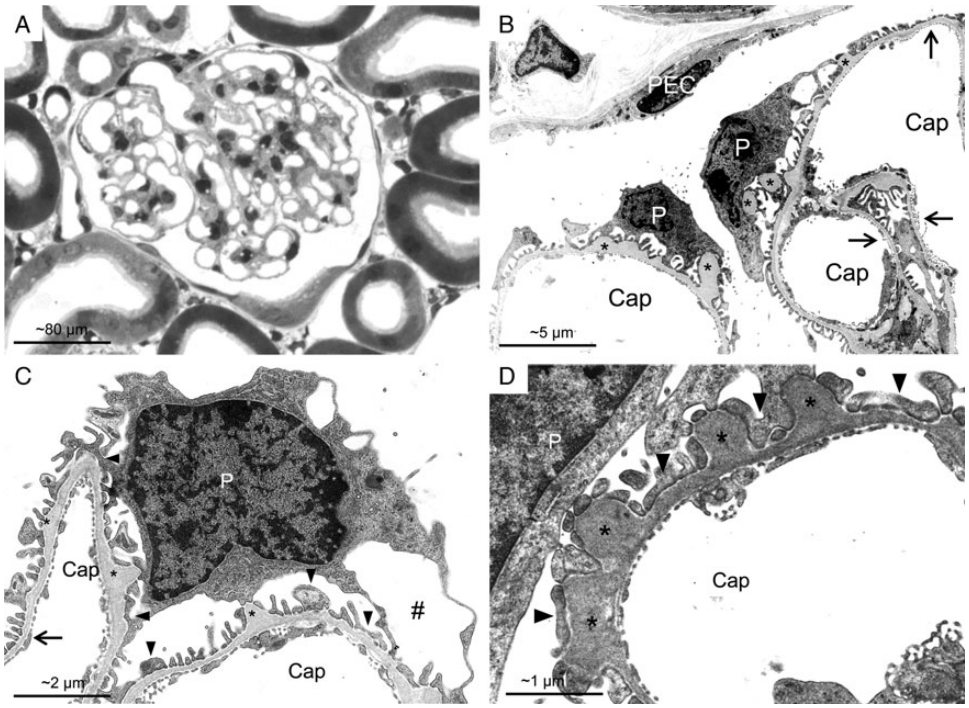

FIGURE 2: Histological analysis of glomeruli from *Slc12a3* knock-out mice. Glomeruli were normal by light microscopy (**A**, Epon semi-thin section stained with methylene blue and azure II). Electron microscopy of glomeruli of NCC-deficient mice (**B–D**, Epon ultra-thin section lead citrate and uranyl acetate). Aside regions with normal appearing GBM (arrows), the GBM shows occasionally prominent protrusions (asterisks) that extend towards the attached podocyte (P). The GBM remains completely covered by the foot processes of the podocytes, but in areas with GBM thickenings foot process effacement (arrowheads) is observed. Occasionally, podocytes show the formation of pseudocysts (hatch) where the foot processes appear to pulled out to thin cell projections. Cap, capillary loop; PEC, parietal epithelial cell. Kidneys of four adult wild-type and four NCC-deficient male mice were analysed.

alterations including heterogeneity and disappearance of the foot processes, with decreased expression of nephrin and podocin, have been described in transgenic mice overexpressing renin [12]. Nephrotic proteinuria was also reported in patients with Addison's disease, another condition associated with hyperreninaemia. Renal biopsy revealed focal segmental glomerular sclerosis and nodular deposits of IgM and C3 [13]. Furthermore, a severe, non-apoptotic detachment of podocytes has been described in one case of Gitelman syndrome, also associated with reduced nephrin expression in the kidney [14]. Chronic hypokalaemia may also play a role. Reungjui *et al.* detected mild proteinuria in hypokalaemic rats given a normal or moderately low potassium diet, with or without hydrochlorothiazide. For the same degree of hypokalaemia, focal glomerular injury was more evident in rats treated with hydrochlorothiazide than in those under low potassium diet, which was attributed to secondary aldosteronism resulting from volume depletion [15].

Altogether, these data suggest that the association of Gitelman syndrome and glomerular proteinuria with abnormal findings of the glomerular basal membrane is probably not coincidental. With increasing awareness for rare inherited kidney disorders and increased number of patients, one can predict that rare complications will emerge. Recently, impaired renal phosphate handling [16], secondary hypertension [9, 17] and CKD [17, 18] have been associated with Gitelman syndrome. Patients with Gitelman syndrome should be evaluated for the presence of proteinuria and biopsied in the presence of glomerular proteinuria.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract format. There is no conflict of interest in relation to this manuscript.

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