LMX1B-associated nephropathy that showed myelin figures on electron microscopy

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Abstract

The mutation of LIM homeodomain transcription factor *LMX1B* gene leads to nail–patella syndrome (NPS), which is characterized by dysplastic nails, hypoplastic patellae, iliac horns and nephropathy. The characteristic renal histological finding of NPS nephropathy is irregular thickening of the glomerular basement membrane with patchy lucent areas, including deposits of bundles of type III collagen fibrils revealed by electron microscopy (EM). Fabry disease is a lysosomal storage disorder caused by a deficiency of α -galactosidase A activity, and the characteristic EM finding is a lamellated membrane structure (myelin figures). We present the case of a male with *LMX1B*-associated nephropathy (LAN) who showed focal segmental glomerulosclerosis (FSGS) on light microscopy, and myelin figures and slight deposits of collagen fibrils on EM, without findings of glomerular basement membrane abnormality suggestive for NPS. A 21-year-old Japanese–Brazilian man was admitted to hospital for an investigation of the cause of proteinuria and decreased renal function. A renal biopsy was performed to investigate the cause of renal damage. Fabry disease was initially considered, based on the presence of myelin figures on EM, but since he had normal α -galactosidase A activity, this initial diagnosis was denied, and the patient was subsequently diagnosed with FSGS. At 22 years after that renal biopsy, the patient was incidentally diagnosed with LAN when NM_002316:3c.746G > A:p.(Arg249Gln) *LMX1B* variant was identified in his older brother by a pre-transplantation examination, and the same mutation was confirmed in the patient. Myelin figures revealed by EM might become one of the clues for the diagnosis of LAN.

Keywords Electron microscopy · Fabry nephropathy · Focal segmental glomerulosclerosis · LMX1B-associated nephropathy · Myelin figure · Nail–patella syndrome · Zebra body

Introduction

The mutation of LIM homeodomain transcription factor *LMX1B* gene leads to nail–patella syndrome (NPS), which is characterized by dysplastic nails and elbow(s), hypoplastic or absent patellae, iliac horns, and nephropathy. NPS can lead to nail–patella-like renal disease (NPLRD), which is characterized by isolated nephropathy without extrarenal manifestations. There is a wide variety in the reported incidence of renal disease and the severity of renal symptoms in NPS patients. The characteristic renal histological finding of NPS nephropathy is irregular thickening of the glomerular basement membrane (GBM) with patchy lucent (moth-eaten) areas, including deposits of bundles of type III collagen fibrils revealed by electron microscopy (EM) [1, 2].

Fabry disease is a lysosomal storage disorder caused by a deficiency of α -galactosidase A activity, and its

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characteristic EM finding is a lamellated membrane structure (myelin figures or zebra bodies).

Lei et al. recently reported two families with *LMX1B*-associated nephropathy (LAN) who showed focal segmental glomerulosclerosis on light microscopy and myelin figures and zebra bodies on EM along with NM_002316.3:c.737G>A:p.(Arg246Gln) mutation, and they proposed that LAN should be included in the differential diagnosis when myelin figures or zebra bodies are observed by EM without extrarenal manifestation [3]. After their report, Pinoto e Vairo et al. described a similar case with the same mutation [4]. Herein we present the case of a patient with LAN who showed the histological findings of focal segmental glomerulosclerosis with podocyte myelin figures and missense mutation NM_002316.3:c.746G>A:p.(Arg249Gln) in *LMX1B* gene. Myelin figures and zebra bodies revealed by EM might become one of the clues for the diagnosis of LAN.

Case report

A 21-year-old Japanese–Brazilian man was admitted to our hospital for an investigation of the cause of his massive proteinuria and decreased renal function. This case was described in our previous report [5]. Other than hypertension, he had no general symptoms such as angiokeratoma, acroparesthesia, or hypohidrosis. He had a family history of renal disease: his mother had died of renal failure at the age of 50 years, and his older brother had been treated for chronic glomerulonephritis. On admission, the patient's blood urea nitrogen concentration was 21.8 mg/dL, and the serum creatinine level was 1.55 mg/dL. The levels of the immunoglobulins, C3, C4 and CH50, were within normal limits. Hepatitis B surface antigen, hepatitis C antibody, latex fixation, anti-nuclear antibody, and anti-DNA antibody were negative. The urinalysis showed massive proteinuria (4–8 g/day) without hematuria. After the patient's admission, a renal biopsy was performed to reveal the cause of renal damage.

In the biopsy sample, segmental hyalinosis and/or sclerosis were observed in five of 20 glomeruli on light microscopy [5] (Fig. 1), and no foam cells or vacuolated cells were present in the glomeruli. A deposition of IgM in the area of sclerosis was observed by immunofluorescence microscopy. Electron microscopy showed extensive focal foot process effacement and myelin figures in podocytes and mesangial cells, and the distributions of these were scattered. Podocyte detachment was not prominent (Fig. 2a). Although the EM findings suggested the presence of Fabry disease, the patient was diagnosed with familial focal segmental glomerulosclerosis at that time because his leukocyte α -galactosidase A activity levels were within normal limits (60.3 nmol/mg

Fig. 1 Light microscopy findings. The glomerulus exhibits segmental sclerosis without vacuolated cells. PAS staining, original magnification×300. Source: Ref. [5]

protein/hr [normal range: 49.8–116.4]) Silicon nephropathy and the influence of cationic amphiphilic drugs as the cause of the myelin figures' appearance on EM were also denied, as the patient had no history of silicon exposure or administration of cationic amphiphilic drugs (chloroquine, gentamycin, and amiodarone).

At 22 years after that diagnosis, a comprehensive gene analysis was performed for the patient's older brother to clarify his primary disease before a renal transplantation. The older brother had also been diagnosed with focal segmental glomerulosclerosis without any general symptoms at 35 years old, in Brazil. His pathological finding was a segmental lesion that was observed in one of nine glomeruli on light microscopy and segmental C3 deposition on immunofluorescence microscopy. Since the heterozygous NM_002316.3:c.746>A:p.(Arg249Gln) *LMX1B* variant was identified by a gene analysis in the older brother [6] and our patient had the same mutation, the patient was finally and incidentally diagnosed with LAN at > 22 years after the first renal biopsy. After the diagnosis of LAN was made, the patient's EM findings were retrospectively analyzed, and fibrillar collagen deposits with periodicity were observed in the GBM, in addition to myelin figures and zebra bodies (Fig. 2b). In the patient's physical re-examination, the characteristic general symptoms of NPS such as dysplastic nails, hypoplastic patella, elbow dysplasia, and iliac horns were not observed in the patient or other family members. A genetic analysis for other family members is under consideration.

Fig. 2 Electron microscopy findings. **a** Myelin figures in podocytes were observed, and extensive foot process effacement was also observed but podocyte detachment was not prominent. $\times 2000$. **b** EM shows scattered deposits of collagen fibrils (white arrow) in the glomerular basement membrane space (partially expanded image)

Discussion

We have presented the case of a patient with LAN for whom an EM examination revealed myelin figures, zebra bodies, and type III collagen bundles with periodicity. Several cases of isolated nephropathy caused by *LMX1B* mutation have been reported [7–10]; since those cases were not classified using classical technical terms such as nail–patella syndrome or NPLRD, the new disease concept of LAN was proposed by Harita et al. [11]. Based on their definition, our patient’s disease category appears to be LAN.

The characteristic EM findings of NPS nephropathy are a focal and diffuse irregular thickening of the GBM with electron-lucent areas (moth-eaten appearance) containing type III collagen bundles with periodicity of approx. 65 nm [12], but the histological features of NPLRD do not always exhibit such specific EM findings [7, 10]. As mentioned above, Lei et al. reported two families with LAN whose cases showed myelin figures and zebra bodies and not the unique GBM appearance of NPS on EM. Those patients had a heterozygous missense mutation in *LMX1B* gene NM_002316.3:c.737G>A:p.(Arg246Gln) [3].

After their report, Pinoto e Vairo et al. described a similar case with the same mutation [4]. In general, highly electron-dense multilamellar inclusions of glycosphingolipids (myelin figures and zebra bodies) on EM is a key diagnostic feature of Fabry disease, which is an X-linked inherited lysosomal storage disorder caused by a deficiency of α -galactosidase A [13]. In addition to Fabry disease, other main causes of the appearance of myelin figures are silicon nephropathy, the administration of cationic amphiphilic drugs (chloroquine, gentamycin, and amiodarone), and the presence of other lysosomal storage disorders such as Niemann–Pick disease.

There was no report about myelin figures and zebra bodies in a case of LAN until the 2020 report by Lei et al. [3]. Although our patient’s genotype is different from those of the cases in the Lei et al. and Pinto e Vairo et al. reports, his light microscopy findings (focal segmental glomerulosclerosis) and EM findings (myelin figures in podocytes)

were quite similar to their cases’ findings. As c.737 G>A and c.746 G>A mutations are within the homeodomain of *LMX1B* gene and since patients with *LMX1B* mutation located in the homeodomain have tended to show renal symptoms without skeletal or nail symptoms [11], the myelin figures and zebra bodies revealed by EM might become one of the clues for the diagnosis of LAN.

The precise mechanism underlying the appearance of myelin bodies in LAN is unclear. *LMX1B* is expressed in podocytes and is a key molecule in the maintenance of food-processing functions and kidney development [14]. The proposed mechanisms of Fabry nephropathy are Notch1-mediated inflammatory and fibrogenic responses in podocytes to lyso-Gb3 stimuli [15] and podocyte damage due to a dysregulated autophagy pathway [16]. Moreover, podocyte injury evokes focal segmental glomerulosclerosis [17], which was present in our patient, Lei et al.’s and Pinto e Vairo et al.’s cases. Podocyte injury might thus be one of the causes of myelin figure generation.

In conclusion, we have described a case of LAN with FSGS shown by light microscopy and myelin figures and zebra bodies revealed by EM. The myelin figures and zebra bodies observed using EM might become one of the clues for the diagnosis of LAN. Nephrologists should be aware of the possibility of not only Fabry nephropathy but also LAN when they encounter a patient with isolated nephropathy with myelin figures and zebra bodies in EM findings. The further accumulation of similar cases is necessary to confirm the significance of the presence of myelin figures in LAN.

Declarations

Conflict of interest The authors declare that no conflict of interest exists.

Ethical approval This article does not contain studies with human participants by any of the authors.

Informed consent Informed consent for the details of his case to be published was obtained from the patient.

References

1. Bongers EM, Gubler MC, Knoers NV. Nail-patella syndrome. Overview on clinical and molecular findings. *Pediatr Nephrol*. 2002;17:703–12.
2. Lemley KV. Kidney disease in nail-patella syndrome. *Pediatr Nephrol*. 2009;24:2345–54.
3. Lei L, Oh G, Sutherland S, Avra G, Higgins J, Sibley R, Troxell M, Kambham N. Myelin bodies in LMX1B-associated nephropathy: potential for misdiagnosis. *Pediatr Nephrol*. 2020;35:1647–57.
4. Pinto E, Vairo F, Pichurin PN, Fervenza FC, Nasr SH, Mills K, Schmitz CT, Klee EW, Herrmann SM. Nail-patella-like renal disease masquerading as Fabry disease on kidney biopsy: a case report. *BMC Nephrol*. 2020;21:341. <https://doi.org/10.1186/s12882-020-02012-3>.
5. Kakita T, Shimohata H, Fujita S, Ogawa Y, Nagai M, Hirayama K, Nakamura H, Kobayashi M. A case of focal segmental glomerulosclerosis with myeloid bodies. *Ran Fail*. 2012;34:801–3.
6. Mori T, Hosomichi K, Chiga M, Mandai S, Nakaoka H, Sohara E, Okado T, Rai T, Sasaki S, Inoue I, et al. Comprehensive genetic testing approach for major inherited kidney diseases, using next-generation sequencing with a custom panel. *Clin Exp Nephrol*. 2017;21:63–75.
7. Boyer O, Woerner S, Yang F, Okeley EJ, Linghu B, Gribouval O, Tete MJ, Duca JS, Klickstein L, Damask AJ, et al. LMX1B mutations cause hereditary FSGS without extrarenal involvement. *J Am Soc Nephrol*. 2013;24:1216–22.
8. Isojima T, Harita Y, Furuyama M, Sugawara N, Ishizuka K, Horita S, Kajiho Y, Miura K, Igarashi T, Hattori M, et al. LMX1B mutation with residual transcriptional activity as a cause of isolated glomerulopathy. *Nephrol Dial Transplant*. 2014;29:81–8.
9. Edwards N, Rice SJ, Raman S, Hynes AM, Srivastava S, Moore I, Al-Hamed M, Xu Y, Santibanez-Koref M, Thwaites DT, et al. A novel LMX1B mutation in a family with end-stage renal disease of ‘unknown cause. *Clin Kidney J*. 2015;8:113–9.
10. Konomoto T, Imamura H, Orita M, Tanaka E, Moritake H, Sato Y, Fujimoto S, Harita Y, Hisano S, Yoshiura K, et al. Clinical and histological findings of autosomal dominant renal-limited disease with LMX1B mutation. *Nephrology*. 2016;21:765–73.
11. Harita Y, Kitanaka S, Isojima T, Ashida A, Hattori M. Spectrum of LMX1B mutations: from nail-patella syndrome to isolated nephropathy. *Pediatr Nephrol*. 2017;32:1845–50.
12. Najafian B, Smith K, Lusco MA, Alpers CE, Fogo AB. AJKD atlas of renal pathology: nail-patella syndrome-associated nephropathy. *Am J Kidney Dis*. 2017;70:e19–20.
13. Najafian B, Fogo AB, Lusco MA, Alpers CE. AJKD atlas of renal pathology: Fabry nephropathy. *Am J Kidney Dis*. 2015;66:e35–6.
14. Brughardt T, Kastner J, Suleiman H, Rivera-Milla E, Stepanova N, Lottaz C, Kubitz M, Boger CA, Schmidt S, Groski M, et al. LMX1B is essential for the maintenance of differentiated podocytes in adult kidneys. *J Am Soc Nephrol*. 2013;24:1830–48.
15. Sanchez-Nino MD, Carpio D, Sanz AB, Ruiz-Ortega M, Mezzano S, Ortiz A. Lyso-Gb3 activates Notch1 in human podocytes. *Hum Mol Genet*. 2015;24:5720–32.
16. Liebau MC, Braun F, Hopker K, Weitbrecht C, Bartels V, Muller RU, Brodesser S, Saleem MA, Benzing T, Schermer B, et al. Dys-regulated autophagy contributes to podocyte damage in Fabry’s disease. *PLoS One*. 2013;8:e63506.
17. Nagata M, Kriz W. Glomerular damage after uninephrectomy in young rats. II. Mechanical stress on podocytes as a pathway to sclerosis. *Kidney Int*. 1992;42:148.

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