Clinical course and pathological findings of two late-onset Fabry hemizygous patients including mulberry cell counts after enzyme replacement therapy

Homare Shimohata¹ · Marina Yamashita¹ · Kentaro Ohgi¹ · Hiroshi Maruyama¹ · Mamiko Takayasu¹ · Kouichi Hirayama¹ · Masaki Kobayashi¹

Received: 2 December 2019 / Accepted: 3 March 2020 / Published online: 18 March 2020

© Japanese Society of Nephrology 2020

Abstract

Fabry disease is an X-linked inherited lysosomal storage disorder caused by a deficiency of α -galactosidase A activity, resulting in the intracellular accumulation of globotriaosylceramide and related glycosphingolipids. The phenotypes of Fabry disease in both males and females are grouped into two categories: the classical type and the late-onset type. The classical type shows general symptoms including angiokeratoma(s), acroparesthesia, hypohidrosis, corneal opacity, and gastrointestinal symptoms from an early age. The late-onset type shows cardiac or renal (or both) symptoms from a late age. We present herein the clinical course and pathological findings of two late-onset hemizygous Fabry patients after the initiation of enzyme replacement therapy (ERT), along with their mulberry cell counts during treatment. One patient's case was a renal-variant type without general symptoms; he showed stable renal function and mild proteinuria but little histological improvement with no change in the mulberry cell count during ERT. The other patient had a cardiac-variant type with renal pathological abnormality. He achieved a mild improvement of renal pathological findings, and his mulberry cell count gradually decreased during the treatment. These findings indicate that monitoring the mulberry cell count might help assess the efficacy of ERT, as a renal pathology tool.

Keywords Fabry nephropathy · Late-onset type · Mulberry cell · Podocyte · Renal biopsy

Introduction

Fabry disease is an X-linked inherited lysosomal storage disorder caused by a deficiency of α -galactosidase A activity, which results in the intracellular accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids [1]. The accumulation of Gb3 and globotriaosylsphingosine (lyso-Gb3) in various cells is responsible for the general clinical manifestations of Fabry disease, i.e., acroparesthesia, angiokeratoma(s), hypohidrosis, cornea verticillate that appear in childhood, and renal, cardiovascular, and/or cerebrovascular complications that emerge in adulthood.

The phenotypes of Fabry hemizygous male patients are grouped into two categories: the classical type and the late-onset type. The classical type shows general symptoms from an early age with no or severely reduced ($<1\%$) residual α -galactosidase A activity, and the late-onset type shows cardiac, renal, or both types of symptoms from later ages with higher α -galactosidase A activity compared to the classical type. Nowadays, since lyso-Gb3 has been recognized as a useful biomarker for distinguishing the classical and non-classical types of Fabry disease [2, 3], we can diagnose the Fabry disease types of male patients relatively easily based on the phenotype, the α -galactosidase A activity, and the lyso-Gb3 level.

Fabry nephropathy is one of the prominent features of both classical-type and late onset-type Fabry patients. An early initiation of enzyme replacement therapy (ERT) with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) can prevent the progressive reduction of renal function in Fabry disease [4]. Although there are some reports of improved renal pathological

✉ Homare Shimohata
h-shimo@tokyo-med.ac.jp

¹ Department of Nephrology, Tokyo Medical University
Ibaraki Medical Center, 3-20-1 Chuo, Ami, Inashiki,
Ibaraki 300-0395, Japan

findings—especially the clearance of Gb-3 from podocytes after ERT in children with classical-type Fabry disease [5, 6]—there have been few investigations of the renal pathological findings after ERT in late-onset Fabry patients.

Here we provide the clinical courses of two male patients with late-onset Fabry disease along with their pathological findings obtained before and at 3 years after the initiation of ERT. We describe the course of each patient's mulberry cell count during his ERT in comparison with his pathological findings. To our knowledge, this is the first report providing the renal pathological findings of late-onset Fabry patients after the initiation of ERT along with the mulberry cell count course data.

Case 1

We reported the case of a 36-year-old Japanese male who was diagnosed with late-onset Fabry disease, specifically the renal-variant type [7]. Except for proteinuria, he had no history of general symptoms such as neuropathic pain, angiokeratoma, hypohidrosis, or corneal opacity. Fabry disease was initially suspected, based on the presence of mulberry cells and bodies. Fabry disease was indeed diagnosed based on a renal biopsy and low α -galactosidase A activity in leucocytes (0.9 mol/h/mg protein). The light microscopy examination of the pretreatment renal biopsy specimen revealed that almost all of the glomeruli contained strikingly enlarged and vacuolated podocytes (Fig. 1a). We measured the degree of podocyte Gb-3 deposits semi-quantitatively using the scoring system for

renal pathology in Fabry disease proposed by Fogo et al. [8]. In brief, podocyte vacuolization was scored for 22 glomeruli (0: none, 1: < 25%, 2: 25–50%, 3: > 50% of podocytes showed cytoplasmic vacuoles), and the final average score was calculated in periodic acid-Schiff (PAS)-stained slides. The podocyte inclusion was scored for three glomeruli (0: no deposits, 1: rare small inconspicuous deposits, 2: more frequent small deposits, 4: > 50% of the tuft involved with large expanded deposits), and the final average score was calculated in toluidine blue-stained semi-thin section slides. The patient's pretreatment vacuolization score was 2.9 (Fig. 1a), and the Gb3 inclusion score by light microscopy of toluidine blue staining was 3.7 (Fig. 1b).

Enlarged lysosomes packed with lamellated membrane structures (myelin-like bodies) were detected in podocytes by electron microscopy (Fig. 1c). After the diagnosis of Fabry disease, ERT with recombinant α -galactosidase A (agalsidase-alfa 0.2 mg/kg) was initiated and continued every 2 weeks for 3 years. The patient's clinical course over the 3 years of treatment is illustrated in Fig. 2, and his clinical parameters related to renal function before ERT and at 3 years after ERT are shown in Table 1. The patient's estimated glomerular filtration rate (eGFR) was approx. 90 mL/min/1.73 m² during the 3 years of ERT. His average urine protein level was 0.56 g/g Cre. The lyso-Gb3 level was 25 nmol/L (< 2 nmol/L) before the start of his ERT.

From the beginning of the patient's ERT, we measured the number of urinary mulberry cells by light microscopy on the whole field (WF), and we observed the constant appearance of mulberry cells during the treatment period (Fig. 2).

Fig. 1 Clinical course of Case 1 after the initiation of ERT, continuing for 3 years. *eGFR* estimated glomerular filtration rate, *RBx* renal biopsy, *UP* urine protein, *WF* whole field

Fig. 2 The kidney biopsy samples of Case 1 before ERT and at 3 years after the start of ERT. **a** Light microscopy (LM) at baseline; enlarged and vacuolated podocytes are shown (periodic acid-Schiff stain $\times 200$). **b** Toluidine blue staining at baseline; Gb3 inclusions on podocytes are observed ($\times 200$). **c** Electron microscopy (EM) image at baseline; myelin-like bodies are present in the podocytes. Magnification: $\times 1000$. **d** LM at 3 years after the start of ERT; enlarged and vacuolated podocytes are present, as at baseline (periodic acid-Schiff stain $\times 200$). **e** Toluidine blue staining 3 years after the start of ERT; the same degree of Gb3 inclusions on podocytes at baseline is present ($\times 200$). **f** EM image 3 years after the start of ERT; myelin-like bodies are present in the podocytes. Magnification: $\times 1000$

Mulberry bodies were observed continually during the treatment period, but they were too small to count on the whole field.

At the 3-year post-ERT time point, we conducted a renal re-biopsy to evaluate the pathological improvement, and we observed that unfortunately, the vacuolization score for the average of 22 glomeruli and the Gb3 inclusion score for the average of six glomeruli were at the same levels as those at the first biopsy (analyzed by unpaired *t*-test) (vacuolization score: pre 2.9 vs. post 2.9, $p=0.533$; inclusion score: pre 3.7 vs. post 3.8, $p=0.626$) (Fig. 1d, e). Myelin-like bodies were

also recognized by electron microscopy at the same level in podocytes (Fig. 1f). The lyso-Gb3 level at the time of the second biopsy was 6.03 ng/mL.

Case 2

Our second patient was a 55-year-old Japanese male who was diagnosed with late-onset Fabry disease (specifically the cardiac-variant type) based on low α -galactosidase A activity in leucocytes (1.0 mol/h/mg protein) and Gb-3 deposits on the heart and kidney [9]. The patient's main symptom was cardiomyopathy, but he also showed Fabry-specific renal pathological findings in the first renal biopsy: enlarged and vacuolated podocytes under light microscopy and myelin-like bodies in podocytes shown by electron microscopy (Fig. 3a, c), with no abnormal urinalysis results except for the presence of urinary mulberry cells and bodies. The vacuolization score for the average of 34 glomeruli was 2.9, and the Gb-3 inclusion score for the average of 8 glomeruli was 3.9 (Fig. 3a, b). After the diagnosis of Fabry disease was made, ERT with recombinant α -galactosidase A (agalsidase-beta 1 mg/kg) was initiated and has continued every 2 weeks as of this writing.

The patient's clinical course for the 3 years after the initiation of ERT treatment is illustrated in Fig. 4. His clinical parameters related to renal function before the ERT and at 3 years after ERT are given in Table 1. The eGFR has been flat at approx. 70 mL/min/1.73 m² during the ERT. The urine protein level has fluctuated between 0.1 g/g Cre and 0.7 g/g Cre but has been maintained at < 1 g/g Cre. At the start of the ERT, the patient's lyso-Gb3 level was 16 nmol/L (0.14–0.75 nmol/L). Notably, the number of urinary mulberry cells gradually decreased from 1 year

Table 1 Clinical parameters before and after enzyme replacement therapy (ERT) related to renal function

Renal function parameter	Case 1		Case 2	
	Before ERT	After ERT	Before ERT	After ERT
TP (g/dL)	7.0	6.9	7.3	7
Albumin (g/dL)	4.7	4.4	4.6	4.3
UA (mg/dL)	6.0	5.9	5.7	5.8
BUN (mg/dL)	13.8	22	9.1	13.7
Creatinine (mg/dL)	0.72	0.88	0.89	0.9
Sodium (mmol/L)	142	143	140	141
Potassium (mmol/L)	4.0	4.4	4.8	4.3
Chloride (mmol/L)	108	107	104	104
Calcium (mg/dL)	9.5	9.7	9.1	9.2
Phosphorus (mg/dL)	3.1	3.8	3.2	3.9
eGFR (mL/min/1.73 m ²)	99.4	78.0	69.4	67.9
UP (g/g Cre)	0.61	1.0	0.39	0.48

BUN blood urea nitrogen, eGFR estimated glomerular filtration rate, TP total protein, UA uric acid, UP urine protein

Fig. 3 Clinical course of Case 2 after the initiation of ERT, continuing for 3 years

Fig. 4 The kidney biopsy samples of Case 2 before ERT and at 3 years after the start of ERT. **a** LM image at baseline; enlarged and vacuolated podocytes are shown (periodic acid-Schiff stain $\times 200$). **b** Toluidine blue staining at baseline; Gb3 inclusions on podocytes are observed ($\times 200$). **c** EM image at baseline; myelin-like bodies are present in the podocytes. Magnification: $\times 1000$. **d** LM image at 3 years after the start of ERT; the number of enlarged and vacuolated podocytes is decreased compared to baseline (periodic acid-Schiff stain $\times 200$). **e** Toluidine blue staining 3 years after the start of ERT; Gb3 inclusions on podocyte are decreased compared to baseline ($\times 200$). **f** EM image 3 years after the start of ERT; myelin-like bodies are present in the podocytes. Magnification: $\times 1000$

after the initiation of ERT and stayed low during the latter half of the treatment period (Fig. 4). Mulberry bodies were observed to an extent that is similar to that of Case 1.

At 3 years after the initiation of ERT for Case 2, we conducted a renal re-biopsy to evaluate his pathological findings. Light microscopy observations revealed enlarged and vacuolated podocytes that were slightly decreased in number. The vacuolization score for the average of 13 glomeruli had significantly fallen from 2.9 to 2.5 after ERT ($p = 0.001$)

(Fig. 3d). Although the Gb3 inclusion score for the average of three glomeruli was also significantly decreased in toluidine blue staining from 3.9 to 3.0 ($p = 0.048$) (Fig. 3e), Gb3 was still solidly deposited in podocytes as shown by electron microscopy (Fig. 3f). The lyso-Gb3 level at the time of the patient's second biopsy was 4.08 ng/mL.

Discussion

Fabry nephropathy is one of the representative features of Fabry disease, and inappropriate treatment can lead to a requirement of renal replacement therapy due to end-stage renal disease. The efficacy of ERT for Fabry nephropathy has been evaluated in several studies based the slope of the GFR, and both agalsidase-alfa and agalsidase-beta are considered effective for preventing the reduction of renal function when they are used soon after the emergence of symptoms [10, 11]. Although a renal biopsy is a useful method to assess the efficacy of ERT for Fabry nephropathy, there are few reports of a pathological renal biopsy-based assessment of the efficacy of ERT because of the biopsy's invasive nature. Thus, some Fabry patients have received dialysis therapy without a definitive pathological diagnosis of primary disease [12].

Tondel et al. evaluated the effects of 5 years of ERT in 12 consecutive young classical-type Fabry patients based on their renal biopsy findings. They reported a substantial clearance of podocyte Gb3 inclusions in three patients who were treated with agalsidase-beta (1 mg/kg every other day) for 5 years and one patient who was treated with agalsidase-alfa (0.4 mg/kg every other day) for 4 years and agalsidase-beta

(1 mg/kg every other day) for 1 year [5]. Skrunes et al. described observing an agalsidase dose- and term-dependent clearing of podocyte Gb3 in patients with classic Fabry disease, including adult patients [6]. These previous studies' renal pathological assessments were conducted in younger classical-type patients, and few investigations assessed the efficacy of ERT by a pathological approach in late-onset adult Fabry patients. Our present report details the clinical courses of two late-onset adult Fabry patients after the start of ERT along with a comparison with their pathological assessments.

Both patients showed stable renal function, and their urine protein levels were both < 1 g/g Cre during the ERT. Arends et al. demonstrated that men with classical Fabry disease had a higher risk of having stage 3A chronic kidney disease compared to men with non-classical (late-onset) Fabry disease [13]. The deterioration of the renal function of their late-onset patients seemed to be mild because of slightly higher residual α -galactosidase A activity [13]. In the present study, the patient with a renal variant (Case 1) who was treated with agalsidase-alfa showed no obvious change in podocyte Gb3 depositions on renal biopsy between pre-ERT and 3 years post-ERT by light or electron microscopy. In contrast, Case 2, the cardiac-variant patient treated with agalsidase-beta, achieved a mild reduction of Gb3 depositions on podocytes after 3 years of ERT (light microscopy and toluidine blue staining findings).

The difference between our two patients might be due to the difference in the variant type and/or the degree of the first-incurred renal histological damage. In addition, in light of the results of young classical-type Fabry patients [5, 6], the differing doses of ERT might influence the clearance of Gb3 inclusions of podocytes. In any case, our present observations revealed that it was difficult for two late-onset adult Fabry patients to completely clear Gb3 deposits in podocytes with 3 years of enzyme treatment.

Another remarkable finding of our present analysis is the confirmation of the usefulness of the mulberry cell count for pathological assessments of the efficacy of ERT. Mulberry cells and mulberry bodies have been recognized as a useful tool for detecting various types of Fabry disease at earlier time points [7, 9, 14–16]. Mulberry cells and mulberry bodies are characteristic features of Fabry nephropathy, and they are distinguishable from oval fat bodies and fat particles based on the inner lamellar appearance of mulberry bodies. Although the usefulness of mulberry cells as a diagnostic tool has been demonstrated by many studies, it has been unclear whether these cells are useful for the monitoring of ERT efficacy. Our patients' cases demonstrated for the first time that the mulberry cell count decreases in accord with the histological improvement of renal pathological findings. That is, mulberry cells may become a useful tool for assessing the pathological changes in Fabry nephropathy.

In the present cases, we counted the number of mulberry cells and not that of mulberry bodies in the whole field of an optical microscope. Strictly speaking, a correction of the urine creatinine concentration is necessary to accurately determine the mulberry cell count without the influence of variations each time. Since we observed a trend that was similar to that of non-corrected data after correcting the mulberry cell count by urine creatinine, and since there has been no standard method for counting the mulberry cell number, we used the mulberry cell counts without correction in the whole field was used in our two patients.

Lyso-Gb3 is attracting attention not only as a diagnostic biomarker but also as a treatment biomarker [2, 3]. It had not been known whether the lyso-Gb3 level reflects the pathological changes in Fabry nephropathy, and in the present study since we could not measure the lyso-Gb3 concentration with the same assay system at the first and second renal biopsies, we unfortunately could not compare the lyso-Gb3 levels precisely between the patients' first and second biopsies and thus cannot delineate the relationship between the lyso-Gb3 level and pathological findings. Although it is unclear whether the lyso-Gb3 level reflects renal pathology findings, the mulberry cells may reflect the pathology findings in Fabry nephropathy more accurately than the lyso-Gb3 level, because mulberry cells are thought to be derived from Gb3 deposits of glomerular cells or tubular cells.

We have provided the clinical courses and pathological findings of two late-onset Fabry hemizygous patients along with their mulberry cell counts after the initiation of ERT. Our analyses revealed that a high-dose and long-term agalsidase administration as ERT is necessary to remove the Gb3 deposits on podocytes in late-onset Fabry patients. The mulberry cell count might become a useful pathology assessment tool for evaluating the efficacy of ERT. A further accumulation of cases is necessary to confirm the usefulness of the mulberry cell count as an assessment tool for Fabry nephropathy.

Acknowledgements We thank Kitasato University Medical Center technician Ms. Natsue Ohta, who measured and reported the mulberry cell counts during the patients' follow-up.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from the patients for their cases to be reported.

References

1. Kint JA. Fabry's disease: alpha-galactosidase deficiency. *Science*. 1970;167:1268–9.
2. Sakuraba H, Togawa T, Tsukimura T, Kato H. Plasma lyso-Gb3: a biomarker for monitoring Fabry patients during enzyme replacement therapy. *Clin Exp Nephrol*. 2018;22:843–9.
3. Maruyama H, Miyata K, Mikame M, et al. Effectiveness of plasma lyso-Gb3 as a biomarker for selecting high-risk patients with Fabry disease from multispecialty clinic for genetic analysis. *Genet Med*. 2019;21:44–52.
4. Warnock DG, Thomas CP, Vujkovic B, et al. Antiproteinuric therapy and Fabry nephropathy: factors associated with preserved kidney function during agalsidase-beta therapy. *J Med Genet*. 2015;52:860–6.
5. Tondel C, Bostad L, Larsen KK, et al. Agalsidase benefits renal histology in young patients with Fabry disease. *J Am Soc Nephrol*. 2013;24:137–48.
6. Skrunes R, Tondel C, Leh S, et al. Long-term dose-dependent agalsidase effects on kidney histology in Fabry disease. *Clin J Am Soc Nephrol*. 2017;12:1470–9.
7. Shimohata H, Ogawa Y, Maruyama H, Hirayama K, Kobayashi M. A case of renal variant of Fabry disease diagnosed by the presence of urinary mulberry cells. *Intern Med*. 2016;55:3475–8.
8. Fogo AB, Bostad L, Svarstad E, et al. Scoring system for renal pathology in Fabry disease: report of the International Study Group of Fabry Nephropathy (ISGFN). *Nephrol Dial Transplant*. 2010;25:2168–77.
9. Shimohata H, Maruyama H, Miyamoto Y, Takayasu M, Hirayama K, Kobayashi M. Urinary mulberry cells and mulberry bodies are useful tool to detect late-onset Fabry disease. *CEN Case Rep*. 2017;6:148–51.
10. Germain DP, Waldek S, Banikazemi M, et al. Sustained, long-term renal stabilization after 53 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol*. 2007;18:1547–57.
11. Beck M, Hughes D, Kampmann C, et al. Long-term effectiveness of agalsidase alfa enzyme replacement in Fabry disease: a Fabry outcome survey analysis. *Mol Genet Metab Rep*. 2015;3:21–7.
12. Saito O, Kusano E, Akimoto T, et al. Prevalence of Fabry disease in dialysis patients: Japanese Fabry disease screening study (J-FAST). *Clin Exp Nephrol*. 2016;20:284–93.
13. Arends M, Wanner C, Hughes D, et al. Characterization of classical and nonclassical Fabry disease: a multicenter study. *J Am Soc Nephrol*. 2017;28:1631–41.
14. Nakamichi T, Miyazaki M, Nakayama K, et al. Fabry's disease discovered with chance urinary mulberry cells: a case report. *CEN Case Rep*. 2013;2(4):9–52.
15. Honda T, Komatsu E, Furuse S, Mise N. Fabry disease diagnosed based on the detection of urinary mulberry bodies. *Intern Med*. 2016;55:2903.
16. Onishi R, Kanaoka K, Sugiura J, et al. A cardiac variant of Fabry disease diagnosed with chance urinary mulberry cells. *Intern Med*. 2018;57:2285–8.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.