Foot Process Effacement Is an Early Marker of Nephropathy in Young Classic Fabry Patients without Albuminuria

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Key Words

Podocyte · Child · Fabry disease · Normalalbuminuria

Abstract

In Fabry disease, globotriaocylceramid (GL3) starts to accumulate in kidney cells in utero, and continues to accumulate throughout childhood and adulthood with progressive tissue damage, which may lead to renal failure. **Material and Methods:** Eight children with classical Fabry disease, median age 12 (range 4–16 years) had a renal biopsy performed before the initiation of enzyme replacement therapy (ERT). All patients were normalalbuminuric and had normal GFR. Three patients were re-biopsied after three or five years. **Results:** In all patients, significant GL3-accumulation was found in several types of kidney cells with high amounts of GL3 in the podocytes. Segmental podocyte foot process effacement was shown in all but two patients; no effacement was seen neither in the youngest male patient at 4 years of age nor in a male aged 12. A 12-year-old female patient had normal podocyte foot processes before the start of ERT, but *de novo*

foot process flattening and unchanged high score of podocyte GL3 accumulation were seen in the re-biopsy after three years of ERT (agalsidase alpha 0.2 mg/kg/every other week). Two boys showed worsening of podocyte effacement in kidney biopsy after five years of agalsidase alpha 0.2 mg/kg/eow. **Conclusions:** Podocyte foot process effacement was found in the majority of eight young classical Fabry patients of both genders after the age of 11 years, without clinical signs of Fabry nephropathy. Kidney biopsies are essential in the early diagnosis of nephropathy and in the evaluation of the response to enzyme replacement therapy of early Fabry nephropathy.

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Introduction

Fabry disease is caused by mutations in the gene encoding the alpha galactosidase A enzyme, causing intracellular accumulation of predominantly globotriaocylceramide (GL3) in a variety of cells; this is a slow, pro-

Table 1. Clinical characteristics

Patient	Gender	Age, years	Mutation	α -Galactosidase A activity (μ kat/kg protein) ^a	eGFR/mGFR (ml/min/1.73 m ²) ^e	Albumin-creatinine ratio (mg/mmol) ^f
1	M	4	Y152C	5.7 ^b	eGFR 110	1.1
2	M	11	M1T	2.5 ^c	eGFR 131	0.9
3	M	12	c.1212_1214delAAG	2.4	eGFR 123/mGFR 95	1.6
4	M	12	c.1212_1214delAAG	3.8	eGFR 97/mGFR 103	0.6
5	F	12	L19P	16.5	eGFR 153/mGFR 103	1.2
6	M	13	C382Y	0.1 ^d	eGFR 144	0.7
7	F	14	M267R	11.1	eGFR 118/mGFR 90	1.2
8	M	16	c.1212_1214delAAG	8.6	eGFR 95/mGFR 112	1.3

^a Reference values α -galactosidase A activity 17.7–26.4 μ kat/kg protein. ^b pmol/punch/h (reference level >32). ^c U (reference level 17–26). ^d nmol/mg protein/h (reference level 50–110). ^e eGFR: GFR estimated with bedside Schwartz formula [12], mGFR measured with iohexol plasma-clearance. ^f Normal reference value albumin-creatinine ratio <2.5 mg/mmol.

gressive process that starts in utero [1]. More than 600 mutations have been identified causing a wide spectrum of classic and non-classic disease as well as non-disease causing variants, and recent publications have highlighted the heterogeneity of the disease in both the genders [2, 3]. The recognition of the increasing prevalence of Fabry disease mutations and diversity of the clinical phenotypes is reflected by the recent reports of observed mutations in 1:3,100 newborns, a much higher frequency than the hitherto traditional accepted number of 1:117,000 affected persons [4, 5]. It follows that increasing focus has been on the identification of relevant biomarkers for the diagnosis of early progressive disease, allowing the initiation of timely enzyme replacement therapy (ERT). It has also been shown that long-term ERT has the capacity to retard or stabilize established nephropathy in adult patients [6]; however, there is increasing evidence that the ability of ERT to prevent organ complications is limited when treatment initiation is delayed until adulthood [7]. On the other hand, early treatment of children has been shown to prevent the progression of nephropathy during 5 years treatment in children with normo- or microalbuminuria [8]. Furthermore, kidney biopsy studies have shown age-dependent progressive accumulation of GL3 in podocytes, and correlations between early kidney damage and albuminuria [9]. Kanai et al. [10] reported a 13-year old male with the C382Y mutation and acroparesthesias who had podocyte GL3 deposits, podocyte depletion, and foot process effacement in the absence of significant proteinuria. Podocyte accumulation of GL3 and foot

process effacement has also been reported in several young classic Fabry patients without clinically evident kidney involvement [8, 11]. In this study, we have further explored the discrepancy between early morphologic Fabry nephropathy and normal clinical kidney findings in young classic Fabry patients with some degree of painful small fiber neuropathy (acroparesthesias).

Material and Methods

A kidney biopsy was performed according to local diagnostic routines in 8 patients prior to initiation of ERT; the median age was 12 years (range 4–16 years). Three patients were re-biopsied after three (patient 5, girl 12 years at baseline) and five years (patients 4 and 8, two boys 12 and 16 years at baseline [8]). Clinical data are shown in table 1. The included patients and/or their designee signed informed consent to be included in this case series. Functional renal evaluation was performed by GFR-measurements (iohexol plasma-clearance and/or estimated GFR) and albumin-creatinine-ratio in morning urine samples (median of three consecutive samples) [12]. The kidney biopsies were examined by a nephropathologist performing light microscopy of paraffin-embedded sections stained with periodic acid Schiff stain (PAS) and McDowell fixed plastic embedded toluidine blue semithin sections as well as electron microscopy (EM) of McDowell fixed plastic embedded sections. Semiquantitative scoring (0 to +) of the GL3-accumulation in the endothelial cells and the mesangial cells was undertaken by EM, whereas the GL3-accumulation in the podocytes was scored by the scoring system of the International Study Group of Fabry Nephropathy (ISGFN) [13]. Evaluation of structural damage of the podocytes was performed by EM and segmental podocyte effacement described as present or not present (yes or no).

Table 2. Kidney biopsy findings

Patient (age/sex)	Podocyte GL3 score (PAS) ^a	Podocyte GL3 score (Toluidine) ^b	Segmental podocyte effacement	Mesangial GL3 deposits	Endothelial GL3 deposits	Fibrosis	Vasculopathy
Patient 1 (4/male)	3	4	no	+	+	–	–
Patient 2 (11/male)	3	4	yes	+	+	–	+
Patient 3 (12/male)	3	2.58	no	+	+	–	–
Patient 4 (12/male)*	3	4	yes	+	+	–	+
Patient 5 (12/female)	2.8	2.6	no**	–	–	–	–
Patient 6 (13/male) [#]	N/A	N/A	yes	–	–	–	+
Patient 7 (14/female)	3	4	yes	(+)	+	–	–
Patient 8 (16/male)*	2.9	4	yes	+	+	–	–

^a PAS, periodic acid Schiff stain. ^b Toluidine, semithin sections. Podocyte scoring method ad modum the International Scoring Group of Fabry Nephropathy [13]. [#] Case previously published by Kanai et al. [10]. * Cases previously published by Tøndel et al. [8]. ** Rebiopsy after 3 years of ERT in patient 5 showed segmental podocyte effacement.

Results

Table 1 shows clinical data in 8 clinical classic Fabry disease children (2 females) with normoalbuminuria and normal GFR. All patients suffered from acroparesthesias of variable degree, and renal biopsies were undertaken to characterize the degree of renal tissue damage prior to initiation of ERT. GL3 accumulation in the podocytes was found in all patients as well as in mesangial and glomerular cells in the majority of the patients (table 2). Segmental podocyte foot process effacement was found in all but two patients (table 2); normal foot processes were found in the youngest male patient (4 years of age) and in a male patient aged 12 years (fig. 1, table 2). A female patient aged 12 years had normal podocyte foot processes in the baseline biopsy. The renal biopsy was repeated after three years of ERT (agalsidase alpha 0.2 mg/kg/every other week (eow)) at the age of 15 years, and *de novo* podocyte foot process effacement and unchanged podocyte GL3 accumulation were found in spite of the moderate effect of ERT on acroparesthesias, clinically stable kidney disease with persistent normoalbuminuria (urinary albumin creatinine ratio was 1.2 mg/mmol at baseline and 1.3 mg/mmol after three years of ERT), and normal GFR (both baseline and follow-up iohexol GFR was 103 ml/min/1.73 m²) (fig. 2). There were no GL3 deposits in glomerular endothelial and mesangial cells on either of the biopsies. Two boys aged 12 and 16 years at baseline were re-biopsied after 5 years of agalsidase alpha 0.2 mg/kg/eow, and the findings have previously been published [8]. Both patients showed increased amount of segmental podocyte effacement and no significant change of GL3-score in the

Fig. 1. Normal podocyte foot processes in male, 12 years, patient 3.

podocytes despite clearing of GL3 in the glomerular endothelial and mesangial cells [8]. In this previous study, the podocytes were evaluated by a nephropathologist using a semiquantitative scoring of segmental podocyte effacement (0; normal, (+); limited segmental podocyte effacement, +; segmental podocyte effacement); both patients had an increase in the score from (+) to +.

Discussion

Our findings demonstrate typical findings of GL3 deposits in podocytes in all patients and glomerular mesangial and endothelial cell deposits in the majority of

Fig. 2. Segmental podocyte foot process effacement, female, 15 years, patient 5 (re-biopsy after three years treatment with agalsidase alpha 0.2 mg/kg/eow).

Fig. 3. Fabry disease: accumulation, tissue injury, compromised function (adapted from [17]).

young patients with a classic phenotype with early onset of neuropathic pain. No signs of clinical kidney disease were apparent, neither in male nor female patients. It is well known that mesangial and endothelial cell deposits are reversible and mesangial structural improvements are seen during ERT in many patients irrespective of

ERT regimen [14, 15]. However, the clearance of GL3 deposits alone has not been shown to confer unequivocal long-term prevention or stabilization of Fabry nephropathy [6], clearly pointing to the multifactorial mechanisms involved in progressive nephropathy in adult patients. Moreover, recent studies have shown that ERT may also reduce podocyte GL3 accumulation in a dose-dependent manner in children and adolescents [8], and dose adjustments have been reported to influence the load of deposits also in adults [16]. Importantly, GL3 accumulation in podocytes has been shown to be age-dependent and associated with functional abnormalities, that is, proteinuria, in patients of younger age [9]. These observations strengthen the concept of sequential progressive renal damage in early Fabry disease as suggested by Eng et al. [17] (fig. 3), and gives a plausible rationale for the assessment with renal biopsy of potential reversible glomerular damage in young classically affected Fabry patients.

The most important observation in this case series was the finding of early segmental podocyte foot process effacement in the majority of normoalbuminuric young classic Fabry patients, a finding that has been previously reported only in a few cases [8, 10, 11]. Intact podocytes are essential in the preservation of the glomerular filtration barrier, and flattening of the foot processes is generally associated with proteinuric glomerular diseases. An increasing number of signaling pathways have been reported to lead to changes in the podocyte cytoskeleton and podocyte injury with foot process effacement and proteinuria [18]. Effacement has been described as a marker for podocyte stress and injury, even in the absence of significant proteinuria [19]. It is not known whether foot process damage in Fabry disease is secondary to GL3 accumulation and/or associated with specific or a number of unspecific signaling pathways or local inflammatory responses. Our findings indicate that early foot process damage may in fact represent a 'silent window' where segmental flattening precedes the development of overt proteinuria in the early phases of a slowly progressive chronic disease like Fabry disease. Furthermore, our case series showed lack of foot process effacement in the youngest boy (aged 4 years) and a boy and a girl at 12 years of age, while the majority of children above 11 years clearly showed abnormal foot processes. This is consistent with the concept of early progressive tissue and organ damage suggested by Eng et al. [17], and also highlighting the necessity of individual evaluation of histopathologic evidence of progression. Our findings suggest that the appearance of this specific sign may serve as an early pre-

albuminuric marker of progressive Fabry nephropathy. One should keep in mind that biopsy sampling errors may challenge the sensitivity of such a finding, and the assessment of foot process damage is subjective and not yet standardized by international consensus. Therefore, further studies are needed to evaluate the predictive value of this structural damage on longer-term changes in proteinuria and renal function.

A surprising finding was the *de novo* appearance of segmental foot process effacement in the re-biopsy in a 15 year old female patient (#5) after three years of treatment with agalsidase alpha 0.2 mg/kg/eow (table 2, fig. 2). Treatment was initiated because of severe acroparesthesias, which did show moderate improvement during the treatment period. The re-biopsy also demonstrated unchanged podocyte GL3 accumulations despite 3 years of ERT, with a cumulative dosing of 15 mg/kg. Moreover, progression of segmental podocyte effacement was found in two previously described male patients treated for five years with agalsidase alpha 0.2 mg/kg/eow [8]. The persistent podocyte GL3 accumulation in the follow-up biopsies and the progressive foot process damage indicates suboptimal effect of the ERT regimen in these three young patients. These cases also demonstrate that adequate ERT dose-response evaluation has to take into account the potential asynchronous effect of the enzyme on clinical (neuropathy) and different cellular elements. This observation and previous studies demonstrate the important role of kidney biopsies in the baseline and follow-up evaluations of Fabry nephropathy and assessment of response to ERT.

There is a general consensus that ERT should be initiated early in classic Fabry disease patients, and early in-

tervention has been shown to attenuate or prevent progressive nephropathy and other organ complications [7]. However, no consensus exists regarding optimal time for initiation of ERT, and no long-term data have documented the capacity of ERT in preventing nephropathy in children beyond 5 years treatment. Kidney biopsies are currently the most promising method for the diagnosis of early and potentially reversible nephropathy, and diagnostic and prognostic markers defined by the scoring of kidney biopsies permit the assessment of treatment effects. Furthermore, kidney biopsies are essential in the evaluation of additional vascular and interstitial damage and the need for adjunctive renoprotective therapy [8, 13].

In conclusion, our results indicate that segmental flattening of foot processes is seen in the majority of classic Fabry disease patients after the age of about 10 years. This finding may serve as an early marker for progressive nephropathy and an indicator for timely ERT intervention in young classic Fabry disease patients of both genders. Follow-up studies and kidney re-biopsies are essential for the validation of this potential prognostic marker as indicator of longer-term preservation of kidney function.

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