

Nephrol Dial Transplant (2014) 29: 619–625  
doi: 10.1093/ndt/gft452  
Advance Access publication 8 November 2013

# Long-term enzyme replacement therapy is associated with reduced proteinuria and preserved proximal tubular function in women with Fabry disease

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## ABSTRACT

**Background.** Fabry disease is an X-linked lysosomal storage disorder caused by mutations in the *GLA* gene. Deficiency of  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) causes intracellular accumulations of globotriaosylceramide (GL-3) and related glycosphingolipids in all organs, including the kidney, often leading to end-stage renal failure. In women with Fabry disease, accumulation of GL-3 in the glomerular podocytes and other renal cells induces progressive, proteinuric nephropathy, but not as severe as in men. Enzyme replacement therapy (ERT) with recombinant  $\alpha$ -Gal A reduces cellular GL-3 deposits in podocytes and tubular epithelial cells. We have previously shown that  $\alpha$ -Gal A is delivered to these cells by different pathways involving different receptors. This study investigated the long-term changes in albuminuria, estimated glomerular filtration rate (eGFR) and urinary markers of both glomerular and tubular dysfunction in women with Fabry disease treated with ERT.

**Methods.** A retrospective, single centre, cohort study evaluated the long-term association between ERT, albuminuria and eGFR in 13 women with Fabry disease and mild renal involvement. In particular, we analysed the changes in the proteinuric profile, including the glomerular marker IgG, the tubular markers  $\alpha_1$ -microglobulin and retinol-binding protein (RBP), and the shared tubular and glomerular markers albumin and transferrin.

**Results.** ERT was associated with a significant reduction in albuminuria and a relatively stable eGFR. The decrease in albuminuria was paralleled by a decrease in both glomerular and tubular urine protein markers.

**Conclusions.** The data indicate that long-term ERT is associated with a reduction in albuminuria and glomerular and tubular urinary protein markers in women with Fabry disease and mild renal manifestations.

**Keywords:** albuminuria, enzyme replacement therapy, Fabry disease, proteinuria

## INTRODUCTION

Fabry disease is an X-linked lysosomal disorder that results from mutations of the gene (*GLA*) that encodes the lysosomal hydrolase  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) [1]. The enzymatic defect leads to progressive lysosomal accumulation of globotriaosylceramide (GL-3) and related glycosphingolipids in the kidney and other tissues [1, 2]. In classically affected males, clinical onset occurs in childhood or adolescence and is characterized by several symptoms [1, 3, 4]. Glycosphingolipid accumulates over time leading to kidney failure, cerebrovascular manifestations, heart failure and eventually premature death [5].

Nephropathy is a dominant feature in Fabry disease and impairment of renal function occurs due to progressive accumulation of GL-3 in renal endothelial, interstitial, tubular epithelial and glomerular cells [1, 6–10]. Early findings in Fabry nephropathy include isosthenuria and tubular dysfunction [1]. Progressive decline in the glomerular filtration rate (GFR) and proteinuria are detrimental signs of renal dysfunction in Fabry disease that may eventually lead to end-stage renal failure [11–14]. This usually occurs in the third to fifth decade of life, when the lysosomal GL-3 accumulation is

irreversible [8, 13]. Fabry nephropathy is generally less severe in women than in men [15]. However, many heterozygous female individuals may be affected similarly to hemizygous male individuals due to random X-chromosomal inactivation [5, 16–18].

Currently, enzyme replacement therapy (ERT) is the only specific treatment for Fabry disease patients [9, 14, 19–23], which has been shown to reduce the GL-3 deposits from multiple cell types. Several studies have suggested that ERT with recombinant  $\alpha$ -Gal A stabilizes or slows the progression of Fabry nephropathy in Fabry disease patients [22, 24–28]. We have previously demonstrated that recombinant  $\alpha$ -Gal A is taken up by the endocytic receptors, megalin and mannose-6-phosphate receptor (M6PR) in the proximal tubule cells [29], and megalin, M6PR and sortilin in the podocytes [30], and by M6PR and sortilin in the glomerular endothelial cells [31], suggesting that ERT may interact with several different parts of the nephron.

In this study, we investigated the association between ERT in women with Fabry disease and the changes in albuminuria, estimated glomerular filtration rate (eGFR) and urinary markers of both glomerular and tubular dysfunction during a mean follow-up period of 6 years.

## MATERIALS AND METHODS

### Ethics

The study was approved by the Regional Research Ethics Committee and conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all participants.

### Study population

This was a retrospective, single centre, cohort study of 17 Caucasian women with Fabry disease referred to the Danish Fabry Centre, Copenhagen University Hospital, Denmark, at which treatment of Fabry patients in Denmark has been centralised. The study included 13 female patients treated with ERT for >1 year and four untreated female patients, all older than 10 years, referred from 2003 to 2011. The study excluded 18 female patients followed at the centre during the same period, either untreated and/or lacking urine samples or on ERT for <1 year. Fabry disease was confirmed in all patients by *GLA* mutation analysis. All patients underwent regular and systematic examinations for manifestations of Fabry disease as a part of the normal follow-up procedure at the hospital. Patients received agalsidase beta (Fabrazyme®, Genzyme) 1 mg/kg intravenously every second week during the follow-up period. Because of a shortage of Fabrazyme® in 2010/2011, most patients subsequently received agalsidase alfa (Replagal®, Shire) 0.2 mg/kg for the remaining follow-up period.

### Data and sample collection

Relevant clinical data were collected from patient files. Data and urine samples were collected over a period of up to 7 years. Plasma creatinine was measured in a certified laboratory using a standardized Roche Modular enzymatic creatinine

assay. The eGFR was calculated using the CKD-EPI equation [32, 33]. Morning urine samples were collected at baseline and during follow-up. Urine samples were frozen at  $-80^{\circ}\text{C}$  until processed.

### Urine protein analysis

Frozen morning urine samples were analysed for total protein (pyrogallol red method) and creatinine (standardized enzymatic method) on a Roche systems Modular clinical chemistry analyser. The following urinary proteins were measured on a Beckman Coulter Image nephelometry system: albumin, IgG, transferrin, retinol-binding protein (RBP) and  $\alpha_1$  microglobulin ( $\alpha_1\text{M}$ ) [34, 35]. The urinary excretions of all proteins were normalized according to the urine creatinine concentration. Normal reference ranges for IgG, transferrin, albumin,  $\alpha_1\text{M}$ , RBP and total protein excretion are listed in Table 1.

### Statistical analyses

The continuous variables are presented by medians and ranges. The albuminuria and eGFR data were analysed using a random coefficient model with the time as a covariate and ERT as a treatment factor. The albuminuria data were log-transformed based on the inspection of residual plots. A  $P < 0.05$  was considered statistically significant. Data analysis was performed using Stata version 12.1 and the figures were prepared using Adobe Photoshop CS3.

## RESULTS

### Patients

Thirteen ERT-treated and four ERT-naive female patients were included. Patient characteristics are given in Table 2. The ERT treatment was initiated on the day of inclusion and continued until the end of follow-up. The ERT-naive patients were not on ERT prior to inclusion or during follow-up. The included patients revealed little or no clinical signs of kidney disease (Table 2). Nevertheless, renal biopsies from three of the patients demonstrated GL-3 inclusions in the podocytes and tubule epithelial cells suggesting renal involvement (data not shown). No major side effects to ERT were reported and there were no deaths or loss in follow-up.

### Albuminuria and eGFR

There was a slight decrease in eGFR during the follow-up period (Figure 1A). In ERT-treated patients, the average

**Table 1. References for established normal values for urinary protein excretion [34, 35]**

Urinary protein	Normal range (mg/mmol creatinine)
IgG	<1.13
Transferrin	<0.19
Albumin	<2.26
$\alpha_1\text{M}$	<1.36
RBP	<0.08
Total protein	<11.31

**Table 2. Baseline characteristics of the 17 female patients with Fabry disease included in this cohort**

Baseline characteristic	N	Median (range)	
		ERT-treated (N = 13)	ERT naïve (N = 4)
<b>Female Fabry disease patients</b>			
Age at inclusion (years)	17	45 (10–62)	34 (30–66)
eGFR (ml/min per 1.73 m <sup>2</sup> )	17	100 (64–159)	99 (88–124)
Urine albumin to creatinine ratio (mg/mmol)	17	1 (0.2–72)	0.26 (0.23–0.52)
Mean follow-up (years)	17	6 (1–7)	5 (2–6)
		N (% of total, N = 13)	N (% of total, N = 4)
<b>Classic Fabry disease mutations/(reference)</b>			
N34S/[36]	1	1 (7.7)	
G85N/[37]	6	4 (31)	2 (50)
A156T/[38, 39]	7	7 (54)	
N355K/[40]	2	1 (7.7)	1 (25)
G271S/[41]	1	0 (0)	1 (25)
On antihypertensive medication	5	4 (31)	1 (25)

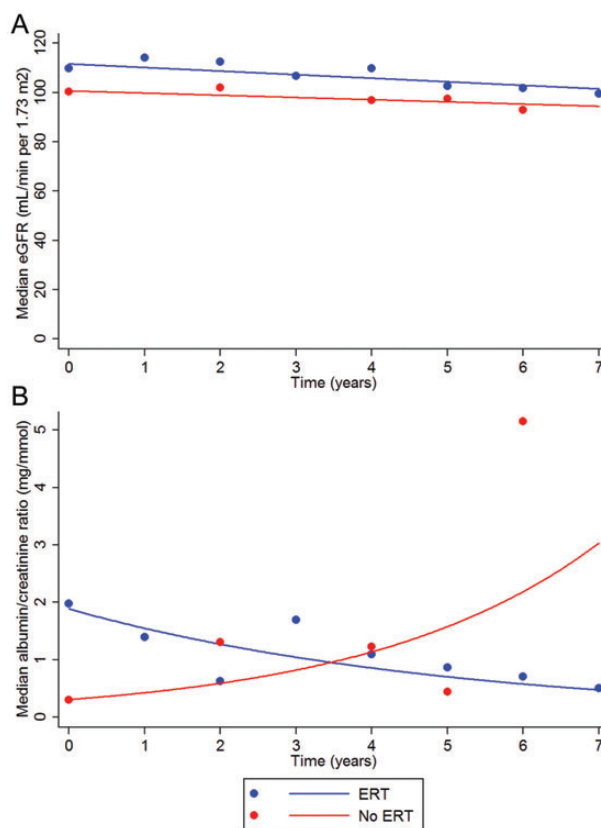
decline in eGFR was 1.45 mL/min/1.73 m<sup>2</sup> per year (95% CI: 0.31–2.58 mL/min/1.73 m<sup>2</sup> per year, P = 0.012) compared with 0.89 mL/min/1.73 m<sup>2</sup> per year (95% CI: –1.51–3.30 mL/min per 1.73 m<sup>2</sup> per year, P = 0.47) in ERT naïve. The decline in the two groups was not significantly different, P = 0.68. During the same period, there was a clearly significant decrease in the albumin/creatinine ratio in the ERT-treated patients (22% per year, 95% CI: 11–34%, P < 0.0001) (Figures 1B and 2). In the ERT-naïve patients, a significant increase was observed (39% per year, 95% CI: 15–67%, P < 0.0001) (Figures 1B and 2). The difference between the two groups was significantly different, P < 0.0001.

### Other urine protein markers

To examine the association between ERT and selective markers of glomerular protein leakage as well as proximal tubule reabsorptive dysfunction, we examined the urinary excretion of IgG, RBP, α<sub>1</sub>M and transferrin, the latter being a marker of both glomerular and tubular dysfunction. Treatment with ERT was associated with a significant decrease in the IgG/creatinine ratio (8% per year, 95% CI: 2–13% per year, P = 0.010) and α<sub>1</sub>M/creatinine ratio (9% per year, 95% CI: 4–14% per year, P < 0.0001, Figure 3). A trend towards lower protein excretion was also observed for the RBP/creatinine ratio (1% per year, 95% CI: –6–7% per year, P = 0.827), transferrin/creatinine ratio (6% per year, 95% CI: –2–13% per year, P = 0.143) and total protein/creatinine ratio (5% per year, 95% CI: –8–19% per year, P = 0.469) in the ERT-treated patients (Figure 3). ERT-naïve patients revealed no significant trend towards an increase in the excretion of glomerular and tubular markers.

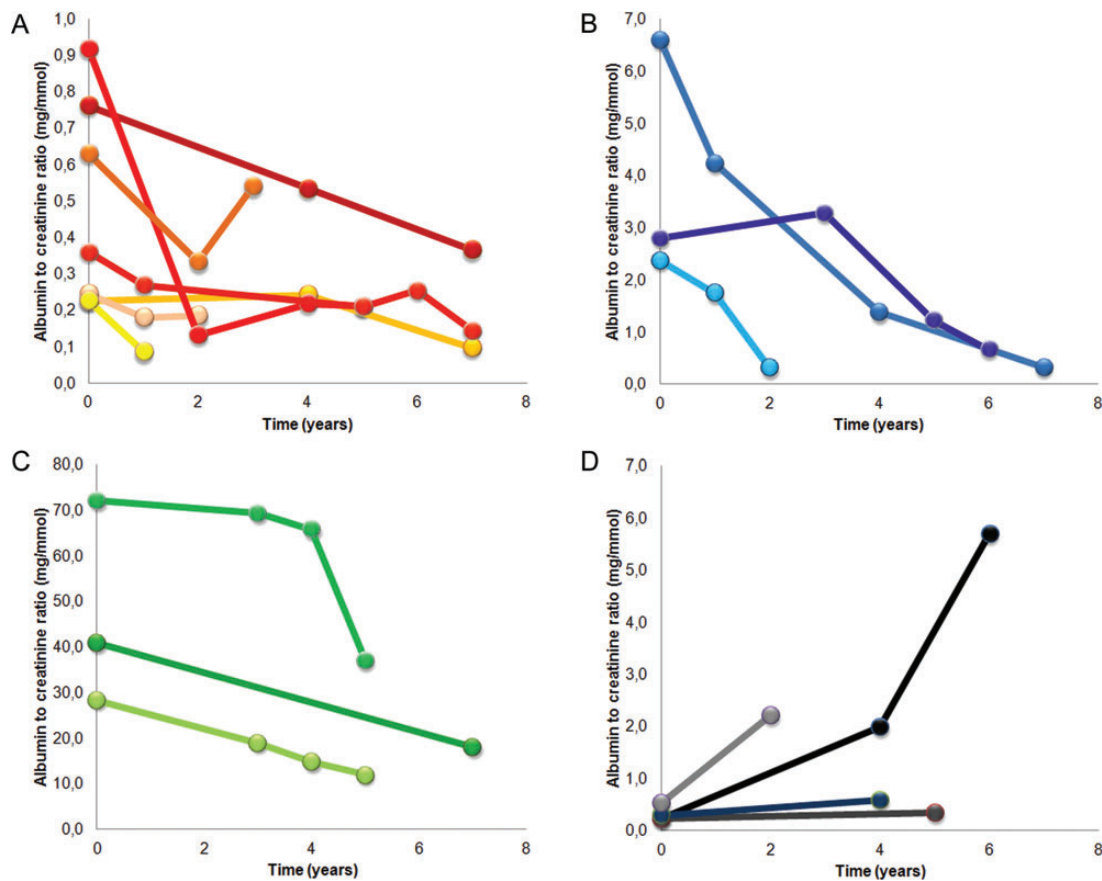
## DISCUSSION

This study shows that in women with Fabry disease and mild renal involvement ERT are associated with stable renal function and a reduction in albuminuria as well as the excretion of both glomerular and tubular protein markers.



**FIGURE 1:** Change in median eGFR (A) and albuminuria (B) in ERT-treated and ERT-naïve female Fabry disease patients during the follow-up period.

The efficacy of ERT is supported by a very recent study showing that long-term ERT with agalsidase alfa stabilized renal function in women with Fabry disease [27]. Several other studies evaluating the effect of short-term ERT with agalsidase beta [24] or agalsidase alfa [26] have shown stabilization of renal function in both male and female Fabry disease patients. The natural progression of Fabry nephropathy has been retrospectively studied in a group of female Fabry disease patients before ERT became available showing a yearly decline in eGFRs of 0.6 ± 2.3 and 2.2 ± 2.2 mL/min/1.73 m<sup>2</sup> in women



**FIGURE 2:** Individual curves showing changes in albuminuria in the 13 ERT-treated patients dependent on the initial level (<2 mg/mmol (A), >2 mg/mmol but <10 mg/mmol (B) and >10 mg/mmol (C)) and in the four ERT-naive patients (D).

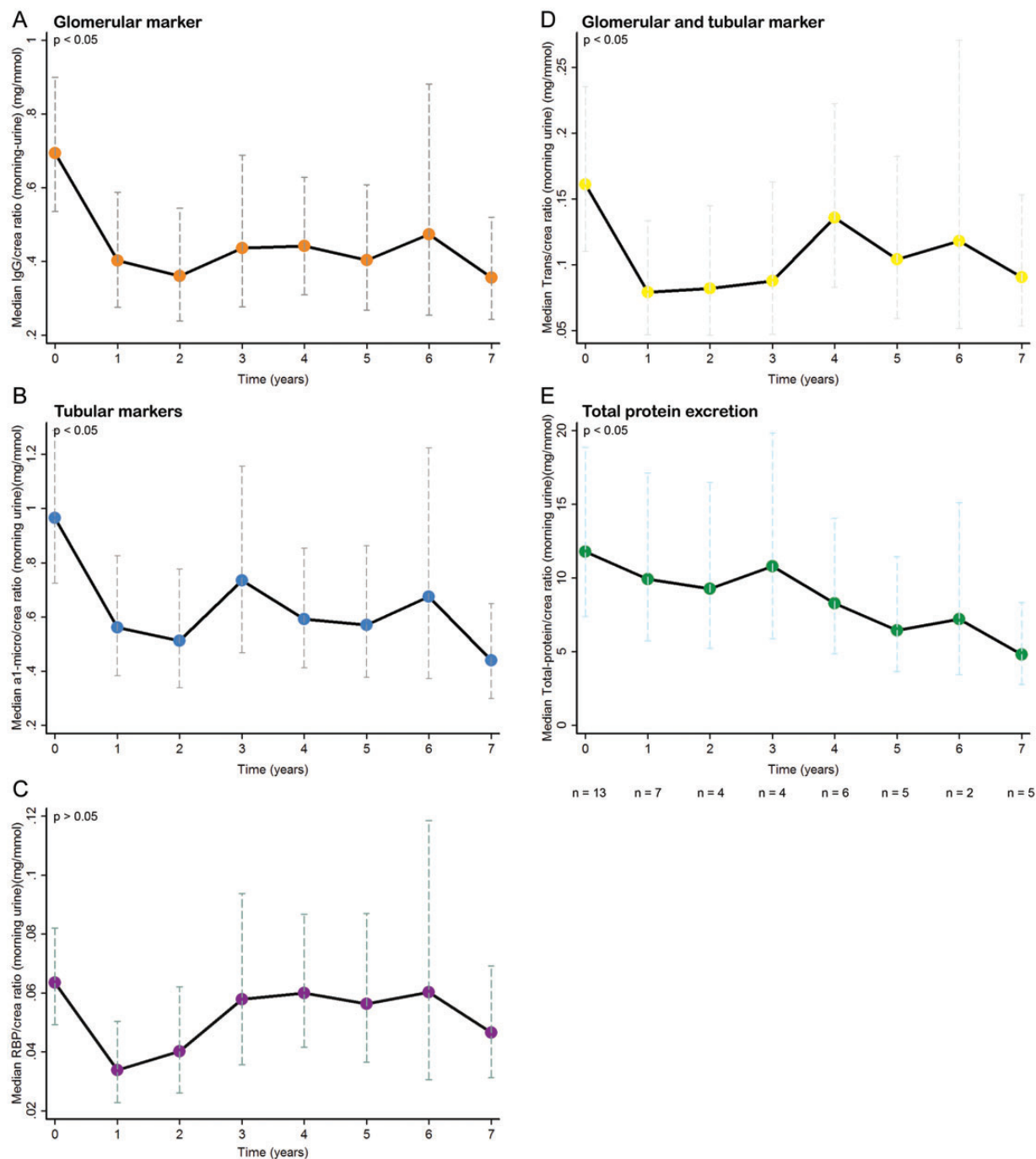
with proteinuria <100 mg/day and between 100 and 1000 mg/day, respectively [11]. Four women with Fabry disease followed at our centre and not receiving ERT also revealed a trend towards increasing albuminuria and declining eGFR during an average of 5-year follow-up.

Proteinuria in Fabry disease patients is associated with progressive tubular injury, interstitial fibrosis and a decline in GFR [42]. Injury to the podocytes due to GL-3 deposits is essential in the development of Fabry nephropathy and proteinuria [9]. We observed GL-3 accumulation in podocytes and tubule epithelial cells in biopsies from three patients included in this study. Recently, it has been shown that ERT is associated with a reduction of GL-3 deposits in podocytes and tubule epithelial cells [9], and that  $\alpha$ -Gal A is taken up by megalin and the M6PR in the proximal tubule cells, [29] and by megalin, M6PR and sortilin in the podocytes [30]. Thus, ERT might exert effects in multiple, different parts of the nephron.

Changes in urinary protein excretion may reflect changes in both glomerular and tubular function. We analysed the pattern of proteinuria in order to gain information on the effect of ERT on the different parts of the nephron [43]. We show that long-term ERT with recombinant  $\alpha$ -Gal A is associated with a significant reduction in albuminuria in women with Fabry disease and mild renal involvement. Furthermore, we observed significant decreases in the excretion of both

glomerular and tubular urine protein markers. The change in the excretion of  $\alpha_1$ M and IgG in ERT-treated patients paralleled the decrease in the excretion of albumin. Thus, this study demonstrates a correlation between changes in the excretion of glomerular and tubular markers, and albuminuria. It may be hypothesized that ERT affects both the podocytes and the tubular epithelial cells, and that uptake of filtered  $\alpha$ -Gal A does not negatively affect proximal tubule function even though significant amounts of recombinant  $\alpha$ -Gal A accumulates here [29].

It is generally recommended to use blockers of the renin-angiotensin system, such as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers (ACEi/ARBs) in proteinuric renal diseases to prevent progression [44, 45]. Although none of the patients had overt proteinuria, 31% of ERT-treated and 25% of ERT-naive patients in our study were treated with ACEi/ARB. Seventy-five percent of the ERT-treated patients on ACEi/ARB initiated ACEi/ARB treatment after being on ERT for 2 years. Thus, the initial and greater decrease in urinary protein excretion after initiation of ERT was independent of ACEi/ARB therapy. Furthermore, a recent study evaluating the long-term use of ACEi/ARB in combination with recombinant  $\alpha$ -Gal A did not show any significant differences in the effect of ERT on eGFR between Fabry disease patients receiving ACEi/ARB and those who did not [27]. Because of the retrospective nature of most studies



**FIGURE 3:** Change in the (median; range) excretion of IgG (A),  $\alpha$ 1M (B), RBP (C), transferrin (D) and total protein (E) during ERT.

involving ACEi/ARB and ERT, no definite conclusion can be made on the potential, additional effects of ACEi/ARB. More systematic studies are needed to evaluate the effect of ERT in combination with other renoprotective medications, and we cannot exclude that the concomitant use of ACEi/ARBs was a possible confounding factor in our study.

The major limitations of this study include the non-randomized design and the lack of a larger control group. Only four female Fabry patients at the study centre with regular follow-up were not receiving ERT making relevant, statistical comparisons impossible. Also, conclusions are restricted by

the low number of study participants. The heterogeneity of the disease in female patients due to skewed X-inactivation also leads to varying degrees of renal pathology; however, deposition of GL-3 was observed in the kidneys of all the three patients biopsied in this study. The change in treatment protocol due to shortage of agalsidase beta may also have influenced the outcome of this study because of the difference in the dosages used: 0.2 mg/kg agalsidase alfa versus 1 mg/kg agalsidase beta. Finally, we used eGFR as endpoint rather than more precise measurements of GFR. The CKD-EPI formula was applied since this formula has been shown to be more accurate

than the MDRD formula for estimating GFR  $\geq 60$  mL/min/1.73 m<sup>2</sup> [32, 46].

In conclusion, long-term ERT with agalsidase beta is associated with a reduction in albuminuria and glomerular and tubular urinary protein markers in women with Fabry disease and mild renal manifestations.

## ACKNOWLEDGEMENTS

The skillful assistance by Hanne Sidemann and study nurse Ira Hagen Pedersen is gratefully acknowledged. This work was supported by the Danish Medical Research Council and Genzyme Corporation.

## CONFLICT OF INTEREST STATEMENT

A postdoc grant for T.P. is in part paid by Genzyme Corporation, Framingham, Massachusetts. U.F.-R. has received unrestricted research grants from Genzyme and serves at the Fabry Registry Advisory Board.

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Received for publication: 25.6.2013; Accepted in revised form: 17.9.2013

*Nephrol Dial Transplant* (2014) 29: 625–635  
doi: 10.1093/ndt/gft458  
Advance Access publication 28 November 2013

## International variation in classification of dialysis withdrawal: a systematic review

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### ABSTRACT

**Background and objectives.** In patients with end-stage renal disease (ESRD), the rate of deaths preceded by dialysis withdrawal is high. However, rates vary across studies and national renal registries. This study aimed to (i) determine how dialysis withdrawal mortality is defined in the literature and (ii) whether mortality rates preceded by dialysis withdrawal change over time.

**Methods.** MEDLINE (1946 to March 2012) and EMBASE (1980 to March 2012) databases were searched. We included epidemiological studies that reported data permitting calculation of crude (unadjusted) mortality rates preceded by dialysis withdrawal. Definitions of dialysis withdrawal were also extracted. Crude mortality rates and 95% confidence intervals were calculated using OpenEpi software. Non-English language studies were excluded.

**Results.** Twenty-three eligible studies were identified; these included 14 527 885 dialysis patients at risk from six countries. Crude mortality rates preceded by dialysis withdrawal ranged from 3 to 50.2 per 1000 person-years. Seven different definitions of dialysis withdrawal were identified, with no assessment of validity. Crude mortality rates preceded by withdrawal have increased over time across the study period 1966 (3 per 1000 person-years) to 2010 (48.6 per 1000 person-years), although these rates are difficult to interpret because of differences in classification. In the USA crude mortality rates preceded by dialysis withdrawal are higher in the older population and have increased over time in the age group 65+ years. In this age group, the crude mortality rate preceded by dialysis withdrawal was 89.4 per 1000 person-years (2008–10) compared with 26.1 per 1000 person-years in the age group 50–64 years (2008–10).

**Conclusion.** Mortality rates preceded by dialysis withdrawal over time should be interpreted with caution because of