Agalsidase Benefits Renal Histology in Young Patients with Fabry Disease

Camilla Tøndel,*† Leif Bostad,‡§ Kristin Kampevold Larsen,‡ Asle Hirth,†‖ Bjørn Egil Vikse,*¶ Gunnar Houge,‖** and Einar Svarstad*¶

*Renal Research Group, Institute of Medicine, University of Bergen, Bergen, Norway; †Department of Pediatrics, ‡Department of Pathology, ‖Institute of Clinical Medicine, ¶Department of Medicine, and **Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital, Bergen, Norway; and §Institute of Gade, University of Bergen, Bergen, Norway

ABSTRACT

The effect of early-onset enzyme replacement therapy on renal morphologic features in Fabry disease is largely unknown. Here, we evaluated the effect of 5 years of treatment with agalsidase alfa or agalsidase beta in 12 consecutive patients age 7–33 years (median age, 16.5 years). We performed renal biopsies at baseline and after 5 years of enzyme replacement therapy; 7 patients had additional biopsies after 1 and 3 years. After a median of 65 months, biopsy findings from all patients showed total clearance of glomerular endothelial and mesangial cell inclusions, and findings from 2 patients showed complete clearance of inclusions from epithelial cells of the distal tubule. The 4 patients who received the highest dose of agalsidase exhibited substantial clearance of podocyte inclusions, and the youngest patient had nearly complete clearance of these inclusions. Linear regression analysis showed a highly significant correlation between podocyte globotriaocylceramide clearance and cumulative agalsidase dose ($r=0.804$; $P=0.002$). Microalbuminuria normalized in five patients. In summary, long-term enzyme replacement therapy in young patients can result in complete globotriaocylceramide clearance of mesangial and glomerular endothelial cells across all dosage regimens, and clearance of podocyte inclusions is dose-dependent.

J Am Soc Nephrol 24: 137–148, 2013. doi: 10.1681/ASN.2012030316

Fabry disease is an X-linked disease affecting glycosphingolipid metabolism due to deficiency of the lysosomal enzyme α-galactosidase A. A hallmark is globotriaocylceramide (GL3) deposits in various cell types; in kidney cells, this is present from early age.[1–3] Fabry disease may cause renal failure, cardiomyopathy, and stroke, all manifestations that can cause serious morbidity and premature death. Kidney disease is almost universal in male patients, and the initial signs are microalbuminuria and proteinuria, which also have been reported in children.[3–5] The causes of nephropathy have not been fully elucidated. Vascular, glomerular, and tubular changes are probably all involved at early stages[1,2] and may also be prominent findings in children despite minimal microalbuminuria or normoalbuminuria.[3]

Since 2001, enzyme replacement therapy (ERT) has been commercially available, and one licensed dose has been recommended for each drug: 0.2 mg/kg every other week for agalsidase alfa and 1.0 mg/kg every other week for agalsidase beta. Initial short-term morphologic studies demonstrated clearance of GL3 in renal endothelial and mesangial cells with agalsidase beta,[6,7] and treatment with agalsidase alfa resulted in decrease of mesangial widening.[8] Partial GL3 clearance of the podocytes has been shown in a limited number of patients after 54 months of

Received March 27, 2012. Accepted October 9, 2012.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Camilla Tøndel, Renal Research Group, Institute of Medicine, University of Bergen, Haukeland University Hospital, N-5021 Bergen, Norway. Email: camilla.tondel@med.uib.no

treatment with agalsidase beta, 1.0 mg/kg every other week,[9] and no studies have evaluated renal morphologic changes after long-term treatment with agalsidase alfa. Studies of treatment effect are generally hampered by small sample sizes and inadequate sensitivity of outcome measures, and no validated biomarkers for early disease progression are available. In pediatric patients, studies have mainly focused on safety and quality of life, and no trials with systematic renal biopsies have been published.[10–13] Early ERT initiation has been suggested to stabilize or improve kidney function. Several reports show limited or no effect of ERT in patients with moderate to severe kidney disease.[9,14–16]

Recently, attempts have been made to achieve a standardized scoring system of Fabry disease–associated kidney lesions with a potential for longitudinal prognostic assessments beyond traditional surrogate markers for disease severity (International Study Group of Fabry Nephropathy).[17] The aim of our study was to evaluate clinical and renal morphologic effects of different ERT doses and drugs given to children and young adults with Fabry disease. Renal biopsies were performed before and after an average of 5 years of ERT. Of note, to our knowledge this is the first systematic renal biopsy study evaluating the long-term effect of different ERT prescriptions on early renal Fabry pathologic features.

Table 1. Baseline characteristics for all patients

Patient No.	Sex/Age (yr)	ACR (mg/mmol)	PCR (mg/mmol)	mGFR (ml/min per 1.73 m^2)	pGL3 (μmol/L)	uGL3 (mol/mol)	α-Galactosidase A Activity (μkat/kg protein)	Mutation
1	M/7	4.8	28.3	106	7.5	3.5	2.40	Missense
2	M/11	3.8	13.0	120	9.3	1.7	2.70	Truncating
3	F/11	6.0	< 0.150 g/L	105	2.6	0.3	16.80	Missense
4	M/12	0.59	9.0	103	8.1	5.0	3.80	Truncating
5[a]	M/16	6.4	11.8	107	11.3[a]	7.4[a]	0.65	Truncating
6	M/16	1.3	10.3	112	13.4	1.8	8.60	Truncating
7[a]	M/17	3.0	10.5	112	9.8[a]	21.6[a]	2.00	Truncating
8	M/17	2.6	8.5	99	9	1.0	2.20	Missense
9	M/18	11.8	28.4	96	4.8	4.5	3.30	Missense
10	M/23	13.6	27.5	113	8.5	3.6	2.50	Truncating
11	M/30	7.2	25.1	86	10.2	8.6	4.90	Truncating
12[b]	M/33	3.6	15.4	111	13.1[b]	1.9	2.30	Truncating

ACR reference: <2.5 mg/mmol; PCR reference: < 20 mg/mmol; mGFR reference: > 90 ml/min per 1.73 m^2; pGL3 reference: 1.6–3.3 μmol/L; uGL3 reference: < 0.6 mol/mol; α-galactosidase A activity (without inhibitor) reference: 17.7–26.4 μkat/kg protein. ACR, albumin-to-creatinine-ratio; PCR, protein-to-creatinine-ratio; pGL3, plasma GL3; uGL3, urine GL3; M, male; F, female.

[a]Patient received agalsidase alfa, 0.2 mg/kg every other week, for 2 years before baseline biopsy; at start of ERT, patient 5 had pGL3 level of 5.1 μmol/L and uGL3 level of 0.73 mol/mol, and patient 7 had pGL3 level of 7.5 μmol/L and uGL3 level of 0.83 mol/mol.

[b]Patient received ERT for 3 years before baseline biopsy (1.5 yr of agalsidase alfa, 0.2 mg/kg every other week, and 1.5 yr of agalsidase alfa, 0.2 mg/kg per week). pGL3 level at start of ERT was 5.9 μmol/L; uGL3 level before start of ERT is not available.

Table 2. Mean baseline data and duration of ERT for all patients and separately for low-dose (group 1) and high-dose (group 2) patients.

Variable	All Patients (n=12)	Group 1 (n=6) (ERT, 0.2 mg/kg Every Other Week)	Group 2 (n=6) (ERT, 0.4 or 1.0 mg/kg Every Other Week)	*P* Value
Patients (n)	12	6	6	
Age (yr)	17.6 (12.7–22.5)	18.2 (10.6–25.7)	17.0 (7.7–26.3)	0.81
α-Galactosidase A activity (μkat/kg protein)[a]	4.3 (1.6–7.1)	4.1 (1.6–6.6)	4.6 (−1.8 to 10.9)	0.26
pGL3 (μmol/L)[a]	8.7 (6.9–10.5)	9.2 (8.3–10.0)	8.2 (4.0–12.4)	0.57
uGL3 (mol/mol)[a]	5.1 (1.4–8.7)	3.6 (0.6–6.6)	6.5 (−1.6 to 14.7)	0.63
ACR (mg/mmol)	5.4 (2.9–7.9)	4.8 (−0.3 to 10.0)	5.9 (2.6–9.3)	0.66
PCR (mg/mmol)	17.0 (11.9–12.1)	15.6 (6.7–24.5)	18.4 (10.0–26.7)	0.20
mGFR (ml/min per 1.73m^2)	105.8 (100.1–111.6)	105.5 (92.8–118.2)	106.2 (100.2–112.2)	0.91
ERT (mo)	60.3 (50.7–70.0)	56.8 (34.2–79.4)	63.8 (60.1–67.6)	0.87

Values are means and 95% confidence intervals in parentheses. Group 1: patients 2, 4, 6, 8, 10, and 11. Group 2: patients 1, 3, 5, 7, 9, and 12. ACR reference: <2.5 mg/mmol; PCR reference: < 20 mg/mmol; mGFR reference: > 90 ml/min per 1.73 m^2; pGL3 reference: 1.6–3.3 μmol/L; uGL3 reference: < 0.6 mol/mol; α-galactosidase A activity (without inhibitor) reference: 17.7–26.4 μkat/kg protein. pGL3, plasma GL3; uGL3, urine GL3; ACR, albumine-to-creatinine-ratio; PCR, protein-to-creatinine-ratio.

[a]All α-galactosidase A and GL3 values are from before start of ERT.

RESULTS

Patients and ERT

Twelve patients, 11 male and 1 female, with a median age of 16.5 years (range, 7–33 years), were enrolled in the study. Two patients were index patients and received a diagnosis because of severe acroparesthesias (patient 5 and patient 8).[18] The remaining patients were identified by family follow-up. Baseline demographic data for all patients are shown in Tables 1 and 2. ERT was given for a median of 65 months (range, 13–69 months) (Table 3). At the time of the final biopsy, 8 patients had received ERT for 5 years, 1 patient for 1 year (patient 8), 2 patients (patients 5 and 7) for a total of 7 years (including 2 years before study start), and 1 patient (patient 12) for a total of 8 years (including 3 years before study start). Total median duration of ERT was 65 months (range, 13–99 months). There were no significant differences in baseline data between the two treatment groups (Table 2) (see Concise Methods for definition of groups).

Clinical Score

The Fabry disease severity score (DS3) at baseline and at biopsy time points are shown in Table 3.[19] The renal DS3 score was low (0–1) in all but one patient (patient 11) at baseline and was stable or improved in all but one patient (patient 7; albuminuria/proteinuria) during the study. The cardiac DS3 score was normal in all patients except patients 5 and 12 (left ventricular hypertrophy) and remained unchanged in all patients

Table 3. DS3 score,[19] ERT, and dosages at times of renal biopsies

Patient No.	Age (yr)/Sex	Time (yr)	DS3 Score					ERT	Duration of ERT (mo)	Cumulative Enzyme Dose (mg/kg)
			PNS	Renal	Cardiac	CNS	Clinical			
1	7/M	0	12	1	0	0	13	Beta, 1.0 mg/kg EOW 0–5 yr	0	
		5	4	0	0	0	4		65	130
2	11/M	0	12	1	0	0	13	Alfa, 0.2 mg/kg EOW 0–5 yr	0	16
		3	11	0	0	0	11		40	27.6
		5	6	0	0	0	6		69	
3	11/F	0	10	1	0	1	12	Beta, 1.0 mg/kg EOW 0–5 yr	0	
		5	1	0	0	1	2		60	120
4	12/M	0	6	0	0	0	6	Alfa, 0.2 mg/kg EOW 0–5 yr	0	26
		5	1	0	0	0	1		65	
5	16/M	−2	12	0	0	0	12	Alfa, 0.2 mg/kg EOW −2 to 0 yr	26	10.4
		0[a]	11	1	5	0	17	Alfa, 0.4 mg/kg EOW 0–4 yr	41	21.6
		1	4	0	5	0	9	Beta, 1.0 mg/kg EOW 4–5 yr	91	77.2
		5	4	0	13	0	17			(66.8)
6	16/M	0	12	0	0	0	12	Alfa, 0.2 mg/kg EOW 0–5 yr	0	
		3	6	0	0	0	6		40	16
		5	6	0	0	0	6		65	26
7	17/M	−2	12	0	0	1	13	Alfa, 0.2 mg/kg EOW −2 to 0y	26	10.4
		0[a]	11	0	0	1	12	Alfa, 0.4 mg/kg EOW 0–5 yr	41	21.6
		1	6	0	0	1	7		94	64
		5	6	1	0	1	8			(53.6)
8	17/M	0	12	0	0	0	12	Alfa, 0.2 mg EOW 0–1 yr	0	
		1	12	0	0	0	12		13	5.2
9	18/M	0	12	1	0	1	14	Beta, 1.0 mg EOW 0–5 yr	0	
		1	9	1	0	1	11		13	26
		5	9	1	0	1	11		66	132
10	23/M	0	12	1	0	0	12	Beta, 0.2 mg EOW 0–5 yr	0	
		1	4	1	0	0	4		13	5.2
		5	4	1	0	0	4		64	25.6
11	30/M	0	12	5	0	0	17	Alfa, 0.2 mg EOW 0–5 yr	0	
		1	4	5	0	0	9		13	5.2
		5	4	5	0	0	9		65	26
12	33/M	−3	12	0	7	0	19	Alfa, 0.2 mg EOW −3 to 1.5 yr	40	24.8
		0[b]	4	1	7	0	12	Alfa, 0.2 mg per wk −1.5 to 5 yr	99	72
		5	4	0	7	0	11			(47.2)

Cumulative dose is calculated from initiation of ERT. The cumulative dose since baseline biopsy is given in parentheses (patients 5, 7, 12). Time, time in years related to baseline biopsy (0); PNS, peripheral nervous system; M, male; beta: agalsidase beta; Alfa, agalsidase alfa; EOW, every other week; F, female.
[a]Two years of ERT before baseline.
[b]Three years of ERT before baseline.

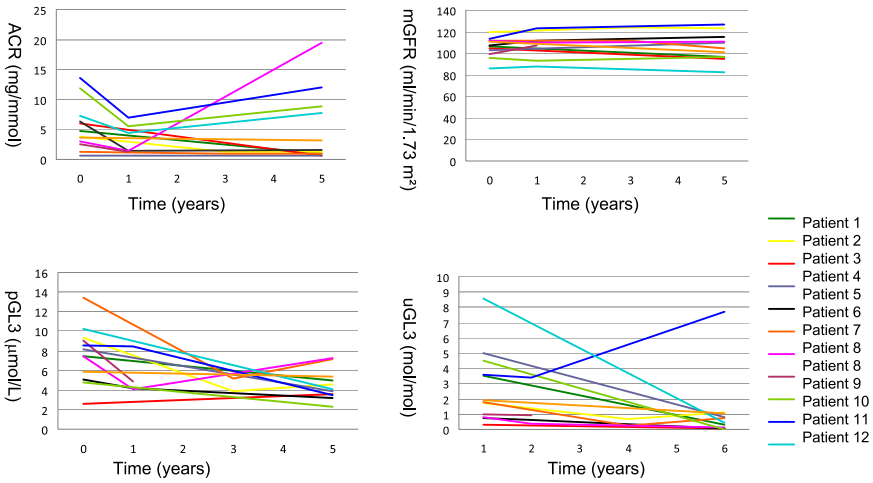

Figure 1. Albumin-to-creatinine-ratio (ACR), mGFR, and GL3 in plasma and urine during 5 years follow-up.

except patient 5. This patient developed significant bradyarrhythmia that required implantation of a pacemaker despite improvement of the renal DS3 score (Table 3), and cardiac structure assessed by echocardiography remained unchanged in the follow-up period.

Kidney Function, Proteinuria, and GL3 Measurements

Baseline and follow-up GFR, proteinuria, and GL3 levels at time of kidney biopsies are shown in Table 1 and Figure 1. All patients had stable measured GFR (mGFR) throughout the observation period, with a mean mGFR of 105.8 and 107.3 ml/min/1.73 m^2 at baseline and after a mean treatment period of 60.3 months (Table 2 and Figure 1). Eleven of 12 patients had CKD stage 1, and 1 patient (patient 11) had CKD stage 2 (Table 1 and Figure 1). Five patients with mild microalbuminuria at age 17 years or younger showed normalization of albuminuria after ERT given for 13–91 months (patients 1–3, 5, and 8), and 2 remained normoalbuminuric (patients 4 and 6). Four patients age 18 years or older had stable microalbuminuria during a treatment period of 59–66 months (Figure 1). Albuminuria and proteinuria did not increase in the study period, with one exception: A 17-year-old male patient (patient 7) had a slight increase in albuminuria and *de novo* occurrence of mild proteinuria after ERT for 94 months (Figure 1). Protein classification showed combined glomerular and tubular proteins in this patient (data not shown). Four patients had slightly elevated urinary protein-to-creatinine ratio (25.1–28.4 mg/mmol) at baseline (Table 1); values returned to normal in 2 of these patients and remained the same in 2. In 45.5% of patients (5 of 11), the urinary GL3 values normalized during treatment, as did plasma GL3 values in 18.2% (2 of 11 patients) (Figure 1). Three patients (patients 5, 7, and 11) received treatment with an angiotensin II receptor antagonist after the baseline biopsy. Despite this renoprotective treatment, the albumin-to-creatinine ratio increased in 1 patient (patient 7), whereas microalbuminuria normalized in 1 patient (patient 5) and remained stable in 1 (patient 11). Anti–α-galactosidase antibodies were found in only 2 patients, and in both cases the finding was transient, with titers just above cutoff values (patients 3 and 5).

Kidney Biopsies

The morphologic changes in the kidney biopsy specimens (obtained *via* light microscopy and electron microscopy) for all patients at baseline and at rebiopsy are shown in Tables 4 and 5. The baseline biopsy samples in all patients showed full score of GL3 inclusions (4.0) and vacuolization (2.9–3.0) in the podocytes despite treatment with agalsidase alfa, 0.2 mg/kg every other week for 2–3 years, in three patients (patients 5, 7, and 12). GL3 inclusions were present in the tubular epithelial cells in all patients and in the mesangial and glomerular epithelial cells in all untreated patients. Mesangial cell inclusions were also found in one patient treated for 2 years with agalsidase alfa, 0.2 mg/kg every other week (patient 5). The rebiopsy findings showed complete clearance of glomerular endothelial cell inclusions and mesangial cell inclusions in all patients after 5 years of ERT, irrespective of treatment regimen (Table 4). This finding was evident also in 7 of 8 patients who had a repeat biopsy after 1–3 years. Complete clearance of inclusions in the epithelial cells in the distal tubuli was found in 2 of 12 patients (patient 1 and 3). The podocytes were almost completely cleared in 1 young male patient (patient 1) (Table 5 and Figures 2 and 3), and 3 patients (patients 3, 5, and 9) showed substantial clearance (Table 5 and Figure 2) after 5 years. Podocyte score decreased in 1 of 8 patients who had a biopsy after 1–3 years, and this patient was treated with agalsidase alfa, 0.4 mg/kg every other week (patient 5). No repeat biopsy showed increased cellular GL3 accumulation. All patients with substantial clearance of the podocytes showed concomitant reduction of podocyte effacement, whereas only 2 patients among those with nearly no change in podocyte GL3 had less podocyte effacement (patients 8 and 11). Regression analysis showed significant correlation between podocyte inclusion clearance and change in albumin-to-creatinine ratio ($r=0.837$; $P=0.001$) (patient 7 excluded because of mixed glomerular and tubular proteinuria) (Figure 4). Nearly identical correlations were found between albumin-to-creatinine ratio and vacuolization score ($r=0.775$; $P=0.005$), as well as composite podocyte score ($r=0.782$; $P=0.003$). Global glomerular sclerosis was present in 5 of 12 patients at baseline and was not visible in 4 of 5 of the rebiopsy samples. No patients showed *de novo* interstitial fibrosis. Arteriopathy was found in 6 of 12 patients at baseline and remained essentially unchanged in the group as a whole. *De novo* arteriopathy emerged in 2 patients (patients 6 and 11) (Table 4).

Table 4. Scoring of renal biopsy specimens (light microscopy and electron microscopy)

Patient No.	Age (yr)/Sex	Time (yr)	GS	FSGS	Glomerular Hyaline	Interstitial Fibrosis (%)[a]	Arteriopathy	Mesangial Cell Inclusion	Glomerular Endothelial Cell Inclusions	Segmental Foot-Process Effacement	Distal Tubule Epithelial Cell Inclusion	Mesangium
1	7/M	0	0/23	−	+	−	−	+	+	+	+	−
		5	0/11	−	+	−	−	−	−	(+)	−	−
2	11/M	0	0/24	−	−	−	−	+	+	(+)	+	−
		3	0/26	−	−	−	−	−	−	(+)	+	−
		5	0/16	−	−	−	−	−	−	(+)	+	−
3	11/F	0	0/29	−	−	−	+	+	+	+	+	Matrix+
		5	0/10	−	−	−	+	−	−	(+)	−	−
4	12/M	0	0/23	−	−	−	+	+	+	(+)	+	−
		5	0/12	−	−	−	−	−	−	+	+	−
5	16/M	−2										
		0[b]	1/12	−	+	5	+	+	−	+	+	Matrix+
		1	1/7	−	−	−	+	−	−	(+)	+	−
		5	0/18	−	−	−	(+)	−	−	(+)	+	−
6	16/M	0	0/22	−	−	−	−	+	+	(+)	+	−
		3	0/15	−	−	−	(+)	−	−	(+)	+	−
		5	0/17	−	−	−	(+)	−	−	+	+	−
7	17/M	−2										
		0[b]	1/9	−	−	5	+	−	−	+	+	−
		1	0/7	−	−	−	+	−	−	+	+	−
		5	0/11	−	−	−	+	−	−	+	+	−
8	17/M	0	0/17	(+)	+	−	+	+	+	+	+	Cells +
		1	0/33	(+)	+	−	+	−	−	−	+	−
9	18/M	0	1/21	−	−	5	−	+	+	+	+	Cells +
		1	0/12	+	−	−	−	−	+	(+)	+	Cells (+)
		5	0/12	+	−	−	−	−	−	−	+	−
10	23/M	0	0/18	−	−	−	−	+	+	+	+	Matrix +
		1	0/9	−	+	−	−	−	−	+	+	Matrix +
		5	0/13	+	+	−	−	−	−	+	+	Matr ix+
11	30/M	0	1/18	−	+	5	−	+	+	+	+	Matrix +
		1	1/23	−	+	5	(+)	−	−	+	+	Matrix +
		5	0/12	−	+	5	+	−	−	(+)	+	−
12	33/M	−3										
		0[c]	1/15	−	−	5	+	−	−	+	+	Matrix+
		5	1/12	−	−	5	+	−	−	+	+	Matrix +

The podocytes are scored separately in Table 5. Plus signs represent clear presence, plus signs in parentheses mean slight presence, and minus signs represent absence. Time, time of biopsy: 0 is baseline, numbers represent years after baseline; GS, global glomerular sclerosis (number of GS/total glomeruli); M, male; F, female.

[a]Estimated semiquantitively and scored to nearest 5%.

[b]Two years of ERT at baseline.

[c]Three years of ERT at baseline.

Table 5. Scoring of podocytes, GL3 inclusions, vacuolization, and composite scores

Patient No.	Age (yr)/ Sex	Time (yr)	Score Osmicated Toluidine Semithin Sections		Score PAS Sections		Composite Score	
			No Scorable Glomeruli	Inclusions (0–4)	No Scorable Glomeruli	Vacuolization (0–3)	No Scorable Glomeruli	Inclusions and Vacuolization (0–7)
1	7/M	0	3	4.0	31	3.0	34	7.0
		5	8	0.13	12	0.25	20	0.4
2	11/M	0	23	4.0	29	2.9	52	6.9
		3	4	4.0	30	3.0	34	7.0
		5	4	4.0	15	2.8	19	6.8
3	11/F	0	3	4.0	32	3.0	35	7.0
		5	15	1.53	12	1.33	27	2.9
4	12/M	0	14	4.0	13	3.0	27	7.0
		5	5	4.0	11	2.64	16	6.6
5	16/M	−2						
		0[a]	3	4.0	10	2.9	13	6.9
		1	4	2.25	10	1.3	14	3.6
		5	6	0.67	16	1.1	22	1.8
6	16/M	0	7	4.0	16	2.88	23	6.9
		3	18	4.0	18	3.0	36	7.0
		5	6	4.0	15	3.0	21	7.0
7	17/M	−2						
		0[a]	3	4.0	9	3.0	12	7.0
		1	21	4.0	9	3.0	30	7.0
		5	4	4.0	14	3.0	18	7.0
8	17/M	0	14	4.0	15	3.0	29	7.0
		1	11	4.0	35	3.0	46	7.0
9	18/M	0	6	4.0	19	3.0	25	7.0
		1	2	4.0	15	3.0	17	7.0
		5	6	2.33	12	2.58	18	4.9
10	23/M	0	4	4.0	16	3.0	20	7.0
		1	23	4.0	13	3.0	36	7.0
		5	1	4.0	16	3.0	17	7.0
11	30/M	0	5	4.0	20	2.85	25	6.9
		1	3	4.0	24	3.0	27	7.0
		5	2	4.0	14	3.0	16	7.0
12	33/M	−3						
		0[b]	26	4.0	16	3.0	42	7.0
		5	30	4.0	12	3.0	42	7.0

Time, time of biopsy: 0 is baseline, numbers represent years after baseline; M, male; F, female.
[a]Two years of ERT at baseline.
[b]Three years of ERT at baseline.

Renal Morphologic Effects of Different ERT Doses

Linear regression analysis showed significant correlation between GL3 clearance of the podocytes and cumulative agalsidase dose ($r=0.804$; $P=0.002$) (Figure 2). Similar correlations were found between cumulative doses and both vacuolization score ($r=0.736$; $P=0.006$) and composite podocyte score ($r=0.783$; $P=0.003$). There were no significant differences in baseline clinical data or months of ERT treatment between the low-dose group (group 1) and the high-dose group (group 2) (Table 2). All patients in both groups achieved complete clearance of glomerular endothelial or mesangial cells (Table 4). Patients with tubular GL3 clearance (patients 1 and 3) were treated with the highest enzyme dose. In contrast to group 1, patients in group 2 had a significant decrease of podocyte inclusions ($P=0.037$). The patients with the highest ERT doses (patients 1, 3, 5, and 9) had significantly higher podocyte inclusion clearance ($P=0.004$) and reduction in albumin-to-creatinine ratio ($P=0.011$) than did group 1 (Table 6).

DISCUSSION

The main finding in this study is the significant correlation between the reduction in podocyte GL3 inclusions and the cumulative dose of agalsidase alfa or beta in young, predominantly male patients (one female patient) treated for a mean of 60.3 months (Figure 2). Essentially no effect on the podocyte inclusions was seen in the low-dose group (group 1; only patient

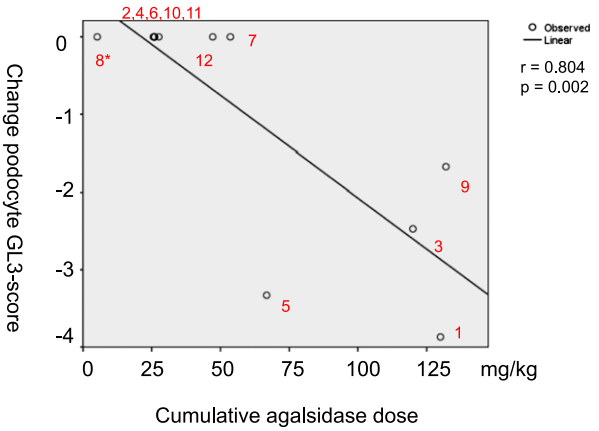

Figure 2. Change of GL3 inclusion score in podocytes in relation to cumulative agalsidase dose after 5 years (linear regression analysis); patients 1, 3, and 9 received agalsidase beta, 1.0 mg/kg every other week; patient 5, agalsidase alpha, 0.4 mg/kg every other week, then a switch to agalsidase beta 1.0 mg/kg every other week after 4 years; patient 7, agalsidase alpha, 0.4 mg/kg every other week; patient 12, agalsidase alpha, 0.2 mg/kg per week; patients 2, 4, 6, 8, and 11, agalsidase alpha, 0.2 mg/kg every other week. Patient 10, agalsidase beta, 0.2 mg/kg every other week. *Patient 8 underwent rebiopsy after 1 year. Numbers in red represent patient numbers.

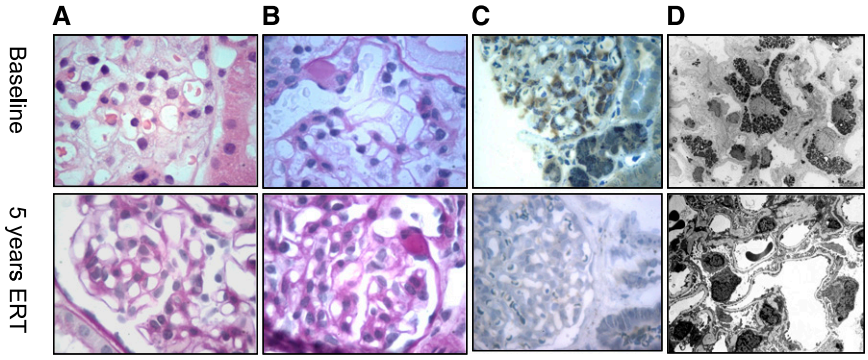

Figure 3. Baseline biopsy specimen (upper panel) shows full score of GL3 deposits. Rebiopsy after 5 years of ERT, 1 mg/kg every other week (lower panel), shows almost complete clearance of deposits in a 7-year-old boy (patient 1). Shown are light microscopic images of hematoxylin and eosin sections (A), PAS sections (B), and osmicated toluidine semithin sections (C) and electron microscopic image (D). Original magnification: × 1000 in A; × 1000 in B; × 400 in C; × 2000 at baseline and × 1500 at 5 years in D.

4 showed marginal reduction), whereas substantial clearance was seen in the 4 patients with the highest doses (patients 1, 3, 5, and 9) (Table 5 and 6). Of note, a dose-response effect on clearance of GL3 after long-term ERT was seen only in podocytes (Figure 2 and Table 4). Irrespective of drug dosage, complete clearance of GL3 inclusions was seen in the glomerular endothelial cells and mesangial cells in all patients after 5 years of ERT; this was also the case in 8 of 9 patients who underwent biopsy after 1–3 years (Table 4).

The clinical significance of podocyte damage in Fabry disease has recently been highlighted by Najafian *et al.* in 14 patients with a median age of 12 years.[20] These authors found significant correlations between proteinuria and podocyte GL3 deposits, as well as foot process effacement, but no correlation between proteinuria and increased endothelial fenestration. There was also correlation between age and the amount of podocyte inclusions. Those findings are corroborated by our observation that all the patients with remarkable clearance of the podocyte inclusions also had decreases in podocyte effacement and microalbuminuria (Tables 4–6 and Figure 4), indicating a relationship between GL3 inclusions and cellular dysfunction. These new findings further expand the concept of relationship between early structural and functional changes and their potential reversibility. Of note, this study and the study by Najafian *et al.* were performed in young patients with normal kidney function, micro- or normoalbuminuria, and low total burden of clinical disease.[20] Furthermore, new receptors involved in dose-dependent agalsidase uptake in human podocytes have recently been described.[21] Taken together, these observations indicate a pivotal role of podocytes in early progression of nephropathy.

Our study demonstrates for the first time a structural and functional rationale for treatment-associated prevention of progressive kidney disease in young patients with Fabry disease who have early nephropathy. We found normalization or stabilization of microalbuminuria in all but one patient after long-term-ERT across different dosing regimens, indicating that the total burden of podocyte damage was also improved or stabilized in nearly all patients. The almost complete GL3 clearance of podocytes in a young patient (patient 1) after 5 years ERT with agalsidase beta, 1.0 mg/kg every other week, is remarkable (Figure 3) and indicates an excellent long-term renal prognosis. Whether this extensive beneficial effect is due to very early treatment, high enzyme dose, or a combination of these remains to be shown.

It is noteworthy that the dose-response effect seemingly is independent of drug type (agalsidase alfa or beta). One patient (patient 5) had remarkable clearance of the podocyte GL3-inclusions after 1 year of treatment with agalsidase alfa, 0.4 mg/kg every other week. However, only marginal effect was seen in patients after treatment with the licensed dose of agalsidase alfa (0.2 mg/kg every other week) (Tables 5 and 6). We did not observe clinical progression of renal disease in either treatment group. Therefore, we cannot exclude that the lower agalsidase dose has a beneficial structural effect on podocytes that cannot be assessed by the scoring method used.[17] However, no renal biopsies were included in previous long-term clinical trials with agalsidase alfa.[22,23] To our knowledge, the current study is the first

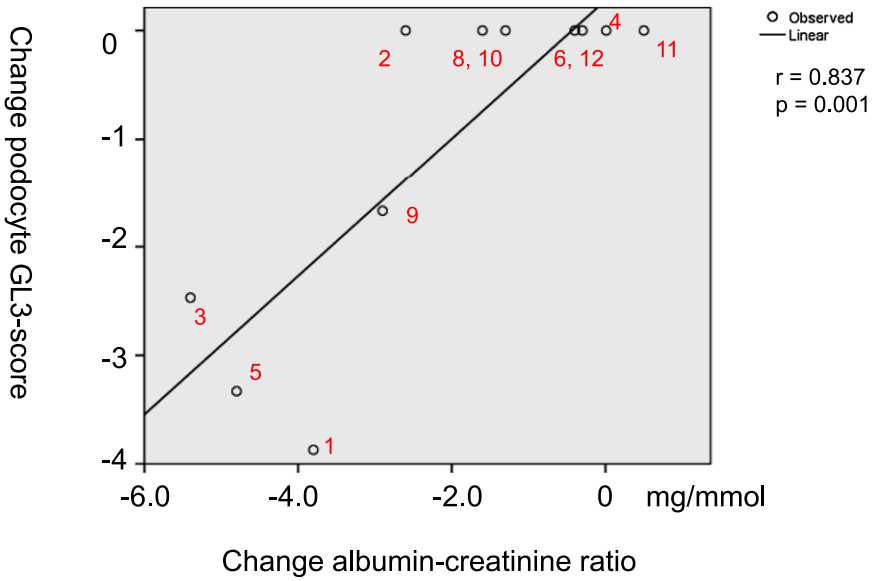

Figure 4. Change of GL3 inclusion score in podocytes in relation to change of albumin-to-creatinine ratio (linear regression analysis). Patient 7 was excluded from the analysis because of mixed glomerular/tubular proteinuria. Numbers in red represent patient numbers.

demonstration that partial GL3 clearance of podocytes is achievable in some patients with standard- or double-dose agalsidase alfa (0.2–0.4 mg/kg every other week), as shown in patients 4 and 5. The current study showing total GL3 clearance of multiple kidney cells after early treatment of young patients with normal kidney function and low total burden of disease supports early initiation of treatment and the use of sufficiently high enzyme dose to achieve maximal clearance of kidney cells.

The importance of early initiation of ERT is still a matter of dispute, and no clear definition of a "window of opportunity" exists in which ERT has been shown to confer future organ protection.[10,12,24] There are few data on monitoring of organ involvement in children, and no clear guidelines exist for initiation of ERT. Previous recommendations and recent studies mainly focus on adult patients and the need of treatment of patients with mild-to-moderate disease,[25,26] at least before clinically advanced disease, such as proteinuria >1 g/d and CKD stage III, is reached.[9,14,16,25,26] In addition, despite treatment with agalsidase alfa or agalsidase beta at the licensed doses, disease progresses in different organs in a subset of patients,[27] as was also observed in a 16-year-old boy in our study who had progression of cardiac disease (need of pacemaker) despite excellent renal effect (patient 5). This indicates that cardiac and kidney disease may respond differently to early ERT, and close collaboration between nephrologists and cardiologists is mandatory in the follow-up of these patients.

Although no large clinical studies have assessed renal progression rate in children, ESRD has been reported as early as 16 years.[28] In a recent pediatric review, Ramaswami *et al.* argue that microalbuminuria, contrary to what is seen in adult patients, seems to be a helpful marker in the evaluation of renal disease in young children.[29] They stress the need of systematic kidney biopsy studies to evaluate potential morphologic biomarkers and treatment effects. Previous and current data provide solid arguments for the recommendation of a baseline kidney biopsy and a follow-up biopsy after about 5 years to ascertain the adequacy of the treatment regimen.[3] Such a follow-up biopsy could potentially indicate the need of a dose increase if failure of cellular clearance or progressive disease is found, or could allow a dose reduction as suggested by Lubanda *et al.*[30] The safety of kidney biopsies, including among children, has recently been confirmed in a large registry study.[31]

Up to now, ERT in Fabry disease has been based on a general "one size fits all" principle, and health authorities have recommended the use of licensed doses of agalsidase beta, 1.0 mg/kg every other week, or agalsidase alfa, 0.2 mg/kg every other week, assuming similar efficacy.[32,33] No studies have documented clinically meaningful dose-response relationships, and we believe our study is the first to document clear dose differences in their effect on renal cellular inclusions, challenging the concept of similarity of the two licensed drug regimens. Hence, our observations support previous reports that suggested similar milligram-to-milligram *in vitro* biocheical potency and clinical effect.[34–38] Our study also supports previous clinical studies that have shown dose-dependent effects on various surrogate endpoints indicating a higher efficiency of agalsidase beta, 1 mg/kg every other week, than agalsidase alfa, 0.2 mg/kg every other week,[23,27,37–41] but further studies are needed to clarify the issue of equipotency of the two currently available drugs.

This study has some limitation. It is an observational case series study, and few patients were studied. Enzyme dose and available ERT drugs were not randomly assigned. Drug treatment was chosen to balance and gain experience with both available drugs, according to our local Fabry disease protocol. One patient was treated with agalsidase beta, 0.2 mg/kg every other week (part of the Dutch head-to-head study).[37] To our knowledge, this is the first longitudinal study to use the International Study Group of Fabry Nephropathy scoring system.[17]

In conclusion, we have demonstrated a dose-dependent clearance of GL3 inclusions in podocytes, but not in endothelial or mesangial cells, in young patients with Fabry disease treated with agalsidase alfa or agalsidase beta for 5 years. Our findings are consistent with the hypothesis that agalsidase alfa and beta have similar biologic activity per milligram. Studies in larger patient cohorts are necessary to confirm these observations.

Table 6. Change of podocyte scores and albumin-to-creatinine ratio after 5 years of ERT

Treatment (agalsidase)	GL3 Inclusion Score (Toluidine Semithin Sections)		Vacuolization Score (PAS Sections)		Composite Score (Inclusions and Vacuolization)		Albumin-to-Creatinine Ratio	
	Change (95% CI)	*P* Value	Change (95% CI)	*P* Value	Change (95% CI)	*P* Value	Change (95% CI)	*P* Value
0.2 mg/kg EOW (6 patients, group 1)	0	1.0	−0.03 (−0.23 to 0.16)	1.0	−0.05 (−0.25 to 0. 15)	0.54	−0.88 (−2.1 to 0.3)	0.12
0.4–1.0 mg/kg EOW (6 patients, group 2)	−1.89 (−3.6 to −0.16)[a]	0.037	−1.1 (−2.3 to 0.08)[a]	0.062	−3.0 (−5.9 to −0.1)[b]	0.044	−0.13 (−8.9 to 8.6)	0.35
1.0 mg/kg EOW (4 patients)	−2.84 (−4.37 to −1.3)[c]	0.010	0.1.66 (−3.18 to −0.14)[c]	0.040	−4.5 (−7.5 to −1.5)[c]	0.018	−4.2 (−6.0 to −2.5)[c] *	0.005

Score reduction is given as mean value with 95% confidence intervals in parentheses. The GL3 inclusions are given in values of 0–4, the vacuolization scores are given in values 0–3, and the combined inclusion and vacuolization scores are given in values of 0–7.[17] Albumin-to-creatinine reference: <2.5 mg/mmol. CI, confidence interval; EOW, every other week.

[a]Group 2 had higher GL3 clearance compared with group 1 ($P=0.022$), as well as greater reduction of vacuolization ($P=0.022$).

[b]Group 2 had borderline higher GL3 clearance compared with group 1 ($P=0.050$).

[c]The subgroup of four patients (patients 1, 3, 5, and 9) with 1.0 mg/kg every other week had greater GL3 reduction in the podocytes ($P=0.004$), greater reduction of vacuolization ($P=0.010$), greater reduction of composite score ($P=0.010$), and greater reduction of albumin-to-creatinine ratio ($P=0.011$) than group 1 (0.2 mg/kg every other week).

CONCISE METHODS

This observational study was conducted in accordance with the Declaration of Helsinki and was approved by the Regional Ethics Committee. Informed consent was in all cases signed by the patient and/or their designees. Patients were included on a consecutive basis in our center. Agalsidase alfa (Replagal, Shire Human Genetic Therapies, Inc., Cambridge, MA) or agalsidase beta (Fabrazyme, Genzyme Corp., Cambridge, MA) was prescribed according to our policy with a balanced use of both drugs. Two patient groups were defined according to enzyme dose. A low-dose group (group 1, $n=6$) received (*1*) agalsidase alfa, 0.2 mg/kg every other week (patients 2, 4, 6, 8, and 11), or (*2*) agalsidase beta, 0.2 mg/kg every other week (patient 10), throughout the study. A high-dose group (group 2, $n=6$) received (*1*) agalsidase beta, 1.0 mg/kg every other week (patients 1, 3 and 9), for the entire study period or (*2*) agalsidase beta, 1.0 mg/kg every other week after 4 years of agalsidase alfa, 0.4 mg/kg every other week (patient 5), agalsidase alfa, 0.4 mg/kg every other week (patient 7), or agalsidase alpha, 0.2 mg/kg, on a weekly basis (patient 12) for the entire study period. Baseline data for the two treatment groups are shown in Table 2, and ERT details are shown in Table 3.

Kidney Biopsies

Our treatment protocol included a baseline biopsy that was done on a consecutive basis before start of ERT in 9 of 12 patients. Three patients had started ERT (agalsidase alfa, 0.2 mg/kg every other week) before the initiation of the current study; their "baseline" biopsy was performed after 2 years of ERT in 2 patients (patients 5 and 7) and after 3 years of ERT in 1 patient (patient 12). Eleven patients had a kidney rebiopsy after 5 years of ERT. Supplementary biopsies were performed after 3 years of ERT in 2 patients and after 1 year in 5 patients to ascertain early morphologic effects and dose adjustments of ERT (Table 3).

Albuminuria and Proteinuria

Urinary albumin-to-creatinine ratio and protein-to-creatinine ratio were measured in 24-hour urine samples until 2005. Since 2005, we have used overnight urine samples recorded as the median value of three consecutive morning samples. An albumin-to-creatinine ratio <2.5 mg/mmol and a protein-to-creatinine ratio <20 mg/mmol are considered normal in our laboratory. Microalbuminuria was defined as an albumin-to-creatinine ratio of 2.5–30 mg/mmol. Urine albumin was analyzed by nephelometry (Behring Nephelometer Analyzer II). Urine protein was analyzed by a turbidimetric method based on benzethonium chloride (Modular Analytics P 800).

Kidney Function Measurements

GFR was measured by an iohexol plasma clearance single-point method after 4 hours (HPLC analysis), calculated according to the method reported by Jacobsson.[42] Body surface area was calculated by the DuBois and DuBois formula in patients age ≥12 years;[43] the distribution volume was calculated according to the method of Stake and Monclair, and body surface area was calculated by the Haycocks formula in the younger patients.[44,45] Estimated GFR was calculated from the serum creatinine using the CKD-Epidemiology formula for

adults or the Schwartz09 formula (a new "bedside" formula) for children.[46,47] Both estimated GFR formulas have recently been validated in patients with Fabry disease.[48,49]

Clinical Disease Activity

The validated disease scoring system Fabry DS3 was used to describe the organ-specific and overall clinical disease burden of the patients.[19]

Renal Morphology

All biopsies were performed and the findings evaluated for representativity through stereomicroscopy by an experienced nephrologist.[50] This evaluation was done immediately after the biopsy was performed. For standard light microscopy, the biopsy tissue was fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin, periodic acid-Schiff (PAS), silver methenamine (Jones stain), and Congo red. Sections from the formalin-fixed, paraffin-embedded material were examined by the immunoperoxidase method for the demonstration of immune deposits. IgG, IgA, IgM, complement 3, and complement 1q antibodies were used. Representative material for electron microscopy was sectioned from the core and immediately fixed in McDowell solution. The tissue was post-fixed in 1% osmium tetroxide and embedded in Epon. Semithin sections were stained with toluidine blue and examined by light microscopy. A representative glomerulus was processed for transmission electron microscopy. The biopsy specimens were scored by light microscopy according to changes in glomeruli (global glomerular sclerosis, FSGS, glomerular hyaline, arteriopathy and arteriolopathy, and interstitial fibrosis). PAS-positive hyaline-like material in the media of small and large arteries was defined as arteriopathy. The GL3 inclusions in mesangial cells, glomerular endothelial cells, and distal tubular epithelial cells were scored as present or not present by light microscopy supplemented by electron micrographs. The GL3 inclusions in the podocytes were scored as values 0–4 by light microscopy of toluidine-stained semithin sections, and vacuolization was scored as 0–3 in standardized PAS-stained sections in line with the scoring system of the International Study Group of Fabry Nephropathy.[17] The mean $\pm$ SD number of scorable glomeruli in PAS-stained sections was 18.8 ± 7.8 at baseline and 15.3 ± 6.4 after five years; in the semithin toluidine blue–stained sections, the respective values were 9.3 ± 8.2 and 6.0 ± 3.9. To have a score based on as many unique glomeruli as possible, a composite score was given as a combination of the GL3 inclusion score and the vacuolization score of the podocytes.

The biopsy samples were scored on a consecutive basis by an experienced nephropathologist (L.B.) blinded for the treatment of the patient. The podocyte GL3 inclusions and vacuolization were scored by a second nephropathologist (K.K.L.) blinded for identity, treatment, and order of the biopsies.

Statistical Analyses

Baseline data, such as age, α-galactosidase A levels, GL3, albumin-to-creatinine ratio, and mGFR, are given as means with 95% confidence intervals. Differences between groups were analyzed with unpaired two-sided *t* tests for normally distributed variables or Mann-Whitney *U* tests for variables not following a normal distribution. Levene tests were used to investigate equality of variances, and corresponding *t* tests were used. Efficacy of treatment was evaluated by paired *t* tests when these followed a normal distribution, and if not, Wilcoxon matched-pairs signed-rank sum tests were used. Linear regression analysis was performed to explore correlations between changes in podocyte GL3 deposits on the one side and cumulative doses, duration of treatment, clinical scores, age, change in albumin-to-creatinine ratio, and change of podocyte effacement. SPSS software, version 17.0 (SPSS Inc., Chicago, IL), was used for processing the data. P values < 0.05 were considered to represent statistically significant differences.

DISCLOSURES

C.T., L.B., A.H., G.H., and E.S. received travel support from Shire and speakers honorarium/travel support from Genzyme. K.K.L. and B.E.V. did not receive any support related to Fabry disease.

REFERENCES

1. Gubler MC, Lenoir G, Grünfeld JP, Ulmann A, Droz D, Habib R: Early renal changes in hemizygous and heterozygous patients with Fabry's disease. *Kidney Int* 13: 223–235, 1978
2. Alroy J, Sabnis S, Kopp JB: Renal pathology in Fabry disease. *J Am Soc Nephrol* 13[Suppl 2]: S134–S138, 2002
3. Tøndel C, Bostad L, Hirth A, Svarstad E: Renal biopsy findings in children and adolescents with Fabry disease and minimal albuminuria. *Am J Kidney Dis* 51: 767–776, 2008
4. Ries M, Ramaswami U, Parini R, Lindblad B, Whybra C, Willers I, Gal A, Beck M: The early clinical phenotype of Fabry disease: A study on 35 European children and adolescents. *Eur J Pediatr* 162: 767–772, 2003
5. Branton MH, Schiffmann R, Sabnis SG, Murray GJ, Quirk JM, Altarescu G, Goldfarb L, Brady RO, Balow JE, Austin Iii HA, Kopp JB: Natural history of Fabry renal disease: Influence of alpha-galactosidase A activity and genetic mutations on clinical course. *Medicine (Baltimore)* 81: 122–138, 2002
6. Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ; International Collaborative Fabry Disease Study Group: Safety and efficacy of recombinant human alpha-galactosidase A—Replacement therapy in Fabry's disease. *N Engl J Med* 345: 9–16, 2001
7. Thurberg BL, Rennke H, Colvin RB, Dikman S, Gordon RE, Collins AB, Desnick RJ, O'Callaghan M: Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy. *Kidney Int* 62: 1933–1946, 2002
8. Schiffmann R, Kopp JB, Austin HA 3rd, Sabnis S, Moore DF, Weibel T, Balow JE, Brady RO: Enzyme replacement therapy in Fabry disease: A randomized controlled trial. *JAMA* 285: 2743–2749, 2001
9. Germain DP, Waldek S, Banikazemi M, Bushinsky DA, Charrow J, Desnick RJ, Lee P, Loew T, Vedder AC, Abichandani R, Wilcox WR, Guffon N: Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol* 18: 1547–1557, 2007
10. Ries M, Clarke JT, Whybra C, Timmons M, Robinson C, Schlaggar BL, Pastores G, Lien YH, Kampmann C, Brady RO, Beck M, Schiffmann R: Enzyme-replacement therapy with agalsidase alfa in children with Fabry disease. *Pediatrics* 118: 924–932, 2006
11. Ramaswami U, Wendt S, Pintos-Morell G, Parini R, Whybra C, Leon Leal JA, Santus F, Beck M: Enzyme replacement therapy with agalsidase alfa in children with Fabry disease. *Acta Paediatr* 96: 122–127, 2007

12. Wraith JE, Tylki-Szymanska A, Guffon N, Lien YH, Tsimaratos M, Vellodi A, Germain DP: Safety and efficacy of enzyme replacement therapy with agalsidase beta: An international, open-label study in pediatric patients with Fabry disease. *J Pediatr* 152: 563–570, 570e1, 2008
13. Schiffmann R, Martin RA, Reimschisel T, Johnson K, Castaneda V, Lien YH, Pastores GM, Kampmann C, Ries M, Clarke JT: Four-year prospective clinical trial of agalsidase alfa in children with Fabry disease. *J Pediatr* 156: 832–837, 837e1, 2010
14. Banikazemi M, Bultas J, Waldek S, Wilcox WR, Whitley CB, McDonald M, Finkel R, Packman S, Bichet DG, Warnock DG, Desnick RJ; Fabry Disease Clinical Trial Study Group: Agalsidase-beta therapy for advanced Fabry disease: A randomized trial. *Ann Intern Med* 146: 77–86, 2007
15. West M, Nicholls K, Mehta A, Clarke JT, Steiner R, Beck M, Barshop BA, Rhead W, Mensah R, Ries M, Schiffmann R: Agalsidase alfa and kidney dysfunction in Fabry disease. *J Am Soc Nephrol* 20: 1132–1139, 2009
16. Breunig F, Weidemann F, Strotmann J, Knoll A, Wanner C: Clinical benefit of enzyme replacement therapy in Fabry disease. *Kidney Int* 69: 1216–1221, 2006
17. Fogo AB, Bostad L, Svarstad E, Cook WJ, Moll S, Barbey F, Geldenhuys L, West M, Ferluga D, Vujkovac B, Howie AJ, Burns A, Reeve R, Waldek S, Noël LH, Grünfeld JP, Valbuena C, Oliveira JP, Müller J, Breunig F, Zhang X, Warnock DG; all members of the International Study Group of Fabry Nephropathy (ISGFN): Scoring system for renal pathology in Fabry disease: Report of the International Study Group of Fabry Nephropathy (ISGFN). *Nephrol Dial Transplant* 25: 2168–2177, 2010
18. Tøndel C, Laegreid LM, Hirth A, Houge G, Månsson JE, Søvik O: [Intravenous enzyme substitution therapy in children with Fabry's disease]. *Tidsskr Nor Laegeforen* 123: 3388–3390, 2003
19. Giannini EH, Mehta AB, Hilz MJ, Beck M, Bichet DG, Brady RO, West M, Germain DP, Wanner C, Waldek S, Clarke JT, Mengel E, Strotmann JM, Warnock DG, Linhart A: A validated disease severity scoring system for Fabry disease. *Mol Genet Metab* 99: 283–290, 2010
20. Najafian B, Svarstad E, Bostad L, Gubler MC, Tøndel C, Whitley C, Mauer M: Progressive podocyte injury and globotriaosylceramide (GL-3) accumulation in young patients with Fabry disease. *Kidney Int* 79: 663–670, 2011
21. Prabakaran T, Nielsen R, Larsen JV, Sørensen SS, Feldt-Rasmussen U, Saleem MA, Petersen CM, Verroust PJ, Christensen EI: Receptor-mediated endocytosis of α-galactosidase A in human podocytes in Fabry disease. *PLoS ONE* 6: e25065, 2011
22. Schiffmann R, Ries M, Timmons M, Flaherty JT, Brady RO: Long-term therapy with agalsidase alfa for Fabry disease: Safety and effects on renal function in a home infusion setting. *Nephrol Dial Transplant* 21: 345–354, 2006
23. Schiffmann R, Askari H, Timmons M, Robinson C, Benko W, Brady RO, Ries M: Weekly enzyme replacement therapy may slow decline of renal function in patients with Fabry disease who are on long-term biweekly dosing. *J Am Soc Nephrol* 18: 1576–1583, 2007
24. Ramaswami U, Parini R, Pintos-Morell G, Kalkum G, Kampmann C, Beck M: Fabry disease in children and response to enzyme replacement therapy: Results from the Fabry Outcome Survey. *Clin Genet* 81: 485–490, 2012
25. Eng CM, Germain DP, Banikazemi M, Warnock DG, Wanner C, Hopkin RJ, Bultas J, Lee P, Sims K, Brodie SE, Pastores GM, Strotmann JM, Wilcox WR: Fabry disease: Guidelines for the evaluation and management of multi-organ system involvement. *Genet Med* 8: 539–548, 2006
26. Warnock DG, Ortiz A, Mauer M, Linthorst GE, Oliveira JP, Serra AL, Maródi L, Mignani R, Vujkovac B, Beitner-Johnson D, Lemay R, Cole JA, Svarstad E, Waldek S, Germain DP, Wanner C; Fabry Registry: Renal outcomes of agalsidase beta treatment for Fabry disease: Role of proteinuria and timing of treatment initiation. *Nephrol Dial Transplant* 27: 1042–1049, 2012
27. Vedder AC, Breunig F, Donker-Koopman WE, Mills K, Young E, Winchester B, Ten Berge IJ, Groener JE, Aerts JM, Wanner C, Hollak CE: Treatment of Fabry disease with different dosing regimens of agalsidase: Effects on antibody formation and GL-3. *Mol Genet Metab* 94: 319–325, 2008
28. Sheth KJ, Roth DA, Adams MB: Early renal failure in Fabry's disease. *Am J Kidney Dis* 2: 651–654, 1983
29. Ramaswami U, Najafian B, Schieppati A, Mauer M, Bichet DG: Assessment of renal pathology and dysfunction in children with Fabry disease. *Clin J Am Soc Nephrol* 5: 365–370, 2010
30. Lubanda JC, Anijalg E, Bzdúch V, Thurberg BL, Bénichou B, Tylki-Szymanska A: Evaluation of a low dose, after a standard therapeutic dose, of agalsidase beta during enzyme replacement therapy in patients with Fabry disease. *Genet Med* 11: 256–264, 2009
31. Tøndel C, Vikse BE, Bostad L, Svarstad E: Safety and complications of percutaneous kidney biopsies in 715 children and 8573 adults in Norway 1988-2010 *Clin J Am Soc Nephrol* 7: 1591–1597, 2012
32. EMA: European Medicines Agency: Assessment report for Fabrazyme. 2010. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Other/2010/11/WC500099241.pdf. Accessed August 1, 2012
33. Linthorst GE, Germain DP, Hollak CE, Hughes D, Rolfs A, Wanner C, Mehta A; European Medicines Agency: Expert opinion on temporary treatment recommendations for Fabry disease during the shortage of enzyme replacement therapy (ERT). *Mol Genet Metab* 102: 99–102, 2011
34. Lee K, Jin X, Zhang K, Copertino L, Andrews L, Baker-Malcolm J, Geagan L, Qiu H, Seiger K, Barngrover D, McPherson JM, Edmunds T: A biochemical and pharmacological comparison of enzyme replacement therapies for the glycolipid storage disorder Fabry disease. *Glycobiology* 13: 305–313, 2003
35. Sakuraba H, Murata-Ohsawa M, Kawashima I, Tajima Y, Kotani M, Ohshima T, Chiba Y, Takashiba M, Jigami Y, Fukushige T, Kanzaki T, Itoh K: Comparison of the effects of agalsidase alfa and agalsidase beta on cultured human Fabry fibroblasts and Fabry mice. *J Hum Genet* 51: 180–188, 2006
36. Blom D, Speijer D, Linthorst GE, Donker-Kopman WG, Strijland A, Aerts JM: Recombinant enzyme therapy for Fabry disease: Absence of editing of human alpha-galctosidase A mRNA. *Am J Hum Genet* 72: 23–31, 2003
37. Vedder AC, Linthorst GE, Houge G, Groener JE, Ormel EE, Bouma BJ, Aerts JM, Hirth A, Hollak CE: Treatment of Fabry disease: Outcome of a comparative trial with agalsidase alfa or beta at a dose of 0.2 mg/kg. *PLoS ONE* 2: e598, 2007
38. Fervenza FC, Torra R, Warnock DG: Safety and efficacy of enzyme replacement therapy in the nephropathy of Fabry disease. *Biologics* 2: 823–843, 2008
39. Smid BE, Rombach SM, Aerts JM, Kuiper S, Mirzaian M, Overkleeft HS, Poorthuis BJ, Hollak CE, Groener JE, Linthorst GE: Consequences of a global enzyme shortage of agalsidase beta in adult Dutch Fabry patients. *Orphanet J Rare Dis* 6: 69, 2011
40. Rombach SM, Dekker N, Bouwman MG, Linthorst GE, Zwinderman AH, Wijburg FA, Kuiper S, Vd Bergh Weerman MA, Groener JE, Poorthuis BJ, Hollak CE, Aerts JM: Plasma globotriaosylsphingosine: Diagnostic value and relation to clinical manifestations of Fabry disease. *Biochim Biophys Acta* 1802: 741–748, 2010
41. Lidove O, West ML, Pintos-Morell G, Reisin R, Nicholls K, Figuera LE, Parini R, Carvalho LR, Kampmann C, Pastores GM, Mehta A: Effects of enzyme replacement therapy in Fabry disease—A comprehensive review of the medical literature. *Genet Med* 12: 668–679, 2010
42. Jacobsson L: A method for the calculation of renal clearance based on a single plasma sample. *Clin Physiol* 3: 297–305, 1983
43. Du Bois D, Du Bois EF: A formula to estimate the approximate surface area if height and weight are known. *Arch Intern Med* 17: 863, 1916

44. Stake G, Monclair T: A single plasma sample method for estimation of the glomerular filtration rate in infants and children using iohexol, I: Establishment of a body weight-related formula for the distribution volume of iohexol. *Scand J Clin Lab Invest* 51: 335–342, 1991
45. Haycock GB, Schwartz GJ, Wisotsky DH: Geometric method for measuring body surface area: A height-weight formula validated in infants, children, and adults. *J Pediatr* 93: 62–66, 1978
46. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration): A new equation to estimate glomerular filtration rate. *Ann Intern Med* 150: 604–612, 2009
47. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL: New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 20: 629–637, 2009
48. Rombach SM, Baas MC, ten Berge IJ, Krediet RT, Bemelman FJ, Hollak CE: The value of estimated GFR in comparison to measured GFR for the assessment of renal function in adult patients with Fabry disease. *Nephrol Dial Transplant* 25: 2549–2556, 2010
49. Tøndel C, Ramaswami U, Aakre KM, Wijburg F, Bouwman M, Svarstad E: Monitoring renal function in children with Fabry disease: Comparisons of measured and creatinine-based estimated glomerular filtration rate. *Nephrol Dial Transplant* 25: 1507–1513, 2010
50. Svarstad E, Iversen BM, Bostad L: Bedside stereomicroscopy of renal biopsies may lead to a rapid diagnosis of Fabry's disease. *Nephrol Dial Transplant* 19: 3202–3203, 2004