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# Enzyme-Replacement Therapy With Agalsidase Alfa in Children With Fabry Disease

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## ABSTRACT

**CONTEXT.** Fabry disease is an X-linked multisystem disorder. Enzyme-replacement therapy in adults has limited efficacy in treating major sequelae of advanced Fabry disease, such as kidney failure or stroke. This prompted a study of the safety and efficacy of enzyme replacement at an earlier stage of Fabry disease.

**OBJECTIVES.** Our purpose with this work was to evaluate safety and to explore efficacy of enzyme treatment with agalsidase alfa in pediatric patients with Fabry disease.

**METHODS.** We conducted a 6-month open-label study at 3 tertiary care centers with 24 children (19 boys and 5 girls) with a mean age of 11.8 (range: 6.5–18) years, to examine safety parameters, including infusion reactions and antiagalsidase alfa antibodies.

**RESULTS.** Agalsidase alfa was well tolerated, and all of the patients completed the study. Six boys and 1 girl had mild-to-moderate infusion reactions. One boy developed transient immunoglobulin G antibodies against agalsidase alfa. The boys showed a significant reduction in plasma globotriaosylceramide on treatment. Mean estimated glomerular filtration rate, cardiac structure, and function were normal and did not change over 26 weeks. Heart rate variability, as determined by 2-hour ambulatory monitoring, was decreased in the boys compared with the girls at baseline. All indices of heart rate variability improved significantly in the boys. Three patients with anhidrosis, as determined by quantitative sudomotor axon reflex testing, developed sweating. Six of 11 patients could reduce or cease their use of antineuropathic analgesics.

**CONCLUSIONS.** Enzyme replacement with agalsidase alfa was safe in this study. The exploratory efficacy analysis documented increased clearance of globotriaosylceramide and improvement of autonomic function. Prospective long-term studies are needed to assess whether enzyme replacement initiated early in patients with Fabry disease is able to prevent major organ failure in adulthood.

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### Key Words

lysosomal storage disease, therapy, stroke, pediatric, cardiac disease

### Abbreviations

FD—Fabry disease  
 GALA— $\alpha$ -galactosidase A  
 Gb<sub>3</sub>—globotriaosylceramide  
 ERT—enzyme-replacement therapy  
 QoL—quality of life  
 eGFR—estimated glomerular filtration rate  
 mean RR—mean beat-to-beat interval  
 SDNN—SD of all normal beat to normal beat intervals  
 SDNN-I—mean of the SD of all filtered RR intervals for all 5-minute segments of the analysis  
 SDANN-I—SD of the means of all filtered RR intervals for all 5-minute segments of the analysis  
 r-MSSD—square root of the mean of the sum of squares of differences between adjacent filtered RR intervals for the length of the analysis  
 pNN50—percentage of differences between adjacent filtered RR intervals that are >50 milliseconds for the whole analysis  
 HUI—Health Utility Index  
 QSART—quantitative sudomotor axon reflex test  
 BPI—brief pain inventory  
 Ig—immunoglobulin  
 LVM—left ventricular mass  
 LVM/h—left ventricular mass indexed for height

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**F**ABRY DISEASE (FD) is a lysosomal storage disease caused by a deficiency of the activity of the lysosomal enzyme  $\alpha$ -galactosidase A (GALA), resulting from mutations of the  $\alpha$ -galactosidase A gene on the Xq22.1 region of the X chromosome.<sup>1,2</sup> So far, 357 mutations have been reported. FD is a panethnic disorder with an estimated prevalence of 1 in 117 000 in males.<sup>3</sup> Female heterozygotes were historically considered to be nonaffected carriers; however, there is increasing evidence that females express signs and symptoms of FD in a delayed manner and in a largely heterogeneous spectrum from no expression to full-blown disease, as seen in hemizygous men.<sup>4-8</sup> The GALA deficiency results in the accumulation of globotriaosylceramide ([Gb<sub>3</sub>] also called ceramidetrihexoside) in vascular endothelial and smooth muscle cells, cardiac myocytes, all types of kidney cells, dorsal root ganglion neurons, neurons of the autonomic nervous system, brain, gastrointestinal tract, and elsewhere.<sup>9-12</sup> Accumulation of Gb<sub>3</sub> is assumed to be responsible for signs and symptoms of FD, including corneal dystrophy, angiokeratomas, neuropathic pain, anhidrosis, left ventricular hypertrophy and valvular abnormalities, kidney dysfunction, and structural and functional abnormalities of the cerebral circulation.<sup>5,9,13-18</sup>

Although signs and symptoms of FD become manifest in childhood, the diagnosis is often delayed, because the early phenotypic expression is heterogeneous and nonspecific. Both the number and severity of symptoms present in a single patient vary widely.<sup>5,7,18,19</sup> Acroparesthesia is a chronic or acute painful burning and tingling sensation in the hands and feet because of the peripheral neuropathy of FD. Reported as sometimes commencing at 3 years in boys and 6 years in girls, the mean age of onset was described as 10 years in boys and 15 years in girls in a cross-sectional study based on questionnaires.<sup>7,15</sup> The neuropathic pain is often dismissed as growth pain, malingering or neurosis, or is misdiagnosed as juvenile rheumatoid arthritis.<sup>7,20</sup> Most subjects with FD have mild-to-severe gastrointestinal symptoms, including abdominal pain, nausea, vomiting, and diarrhea or constipation beginning in adolescence.<sup>7,18,20</sup> Patients with FD have a characteristic progressive pattern of dysmorphic features in the absence of a single defining trait, mainly manifesting in adult hemizygous men without residual enzyme activity.<sup>21</sup>

In male subjects, kidney function begins to deteriorate in the third or fourth decade of life and progresses to end-stage kidney disease requiring kidney dialysis or transplant.<sup>13,22</sup> Although the mean onset of renal, cardiac, and cerebrovascular complications of FD occurs after the age of 20 years, they have been reported in male and female subjects under the age of 20 years.<sup>5,7,18</sup>

Until recently, treatment of FD has been limited to symptomatic management of neuropathic pain and to dialysis and/or kidney transplantation for end-stage re-

nal disease. Recent studies suggest that enzyme-replacement therapy (ERT) may be able to alleviate symptoms and slow or potentially halt the progression of the disease.<sup>23-28</sup> However, possibly because of the irreversible nature of secondary end-organ damage caused by the condition, the efficacy of ERT is limited in adult patients with advanced FD. Deterioration of kidney and heart function has been observed despite enzyme replacement in some patients.<sup>26,29,30</sup> Others developed white matter lesions or strokes, which mandate aggressive, supportive primary and secondary prevention in patients at risk.<sup>26,29-33</sup> Pain control may be incomplete, and the effects of enzyme replacement on quality of life (QoL) were small.<sup>25,29,34,35</sup>

Because of the progressive nature of FD and the general absence of major organ dysfunction in childhood, early intervention with ERT might represent a therapeutic "window of opportunity" and has the potential to provide greater benefit. However, no clinical trials have been performed to demonstrate the safety of ERT in Fabry children. We, therefore, conducted a prospective, multicenter, open-label clinical trial to assess safety and explore the efficacy of agalsidase alfa in a pediatric population with FD.

## METHODS

### Study Design

This study was an open-label, multicenter, 26-week study of agalsidase alfa administered at a biweekly dose of 0.2 mg/kg designed to assess the safety and explore efficacy in pediatric patients of both genders with FD. The study was conducted at the National Institutes of Health, the Center for Lysosomal Storage Diseases at the University of Mainz, and the Hospital for Sick Children. The institutional review board or independent ethics committee at each site approved the study. All of the subjects and their parents or legal guardian agreed to participate and signed written informed consent or assent before enrollment in the study.

### Study Subjects

Male and female children with FD between the ages of 6.5 and 18 years who were naïve to ERT were eligible for this study (Table 1). In boys, FD was confirmed by measurement of deficient GALA activity in peripheral blood white cells, and in symptomatic girls, the disease was confirmed by genetic analysis demonstrating the presence of a disease-causing  $\alpha$ -galactosidase A gene mutation.

### Agalsidase Alfa Treatment

Starting at week 1 and continuing every other week through week 25, subjects received agalsidase alfa (Shire Human Genetic Therapies, Cambridge, MA) at a dose of 0.2 mg/kg delivered biweekly as a 40-minute intrave-

**TABLE 1 Patient Demographics**

Variable	Baseline Characteristics
<i>N</i>	24
Male/female	19/5
Race, <i>n</i>	
White	22
Hispanic	2
Weight, mean ± SE (range), kg <sup>a</sup>	42.1 ± 3.5 (22.5–88.5)
Height, mean ± SE (range), cm <sup>a</sup>	147.3 ± 3.5 (121–179)
Duration of Fabry symptoms, mean ± SE (range), y	4.0 ± 0.9 (0.0–16.1)
Family history of FD	
Yes	22
No	1
Unknown	1
Organ systems involved, <i>n</i>	
1–3	9
3–6	10
>6	5

<sup>a</sup> Height and weight were similar to the normal population of that age.

nous infusion. Agalsidase alfa is manufactured in a genetically engineered human cell line with identical amino acid sequence and glycosylation pattern of native human GALA.<sup>25</sup>

### Safety Assessment

Vital signs, results of physical and neurologic examinations, clinical laboratory measurements, measurements of antiagalsidase alfa antibodies, electrocardiograms, and serious and nonserious adverse events observed by the investigator or reported by the patient were monitored continuously during the study. Serum was assayed for antiagalsidase alfa antibodies at baseline and at weeks 9, 17, and 26 using a plate enzyme-linked immunosorbent assay. A positive response was defined as an absolute absorbance of >0.04 U and a time point/baseline ratio ≥2.0.

### Exploratory Efficacy Evaluations

A synopsis of the explorative efficacy variables is provided in Table 2.

#### *Gb*<sub>3</sub>

Plasma and urine sediment *Gb*<sub>3</sub> levels were measured as described previously.<sup>36</sup>

### Renal Status

Renal function was assessed by serial estimates of glomerular filtration rates (eGFRs) using the Counahan-Barratt equation and by serial measurements of 24-hour urinary albumin excretion.<sup>37</sup>

### Cardiac Status

Cardiac status was determined by echocardiography and electrocardiogram as described previously.<sup>18</sup>

### Heart Rate Variability

Despite the absence of published data on heart rate variability in FD, we hypothesized that involvement of the autonomic nervous system in FD reduces heart rate variability and that ERT could reduce this dysfunction. Heart rate variability was assessed quantitatively from 2-hour ambulatory monitor recordings; measured were mean beat-to-beat interval ([mean RR] milliseconds), SD of the normal beat to normal beat intervals over the length of the analysis (SDNN), mean of the SD of all of the filtered RR intervals for all 5-minute segments of the analysis (SDNN-i), SD of the mean of all filtered RR intervals for all 5-minute segments of the analysis (SDANN-i), square root of the mean of the sum of squares of differences between adjacent filtered RR intervals for the length of the analysis (r-MSSD), and percentage of differences between adjacent filtered RR intervals that are >50 milliseconds for the whole analysis (pNN50). The mean RR interval reflects the mean heart rate during the recording period, and the last 5 parameters describe the magnitude of the deviation of heart rates from the mean RR intervals using standard statistical analyses of the Gaussian distribution of heart rate.

### Diaries

All of the subjects kept daily diaries to record the severity and frequency of neuropathic pain, pain crises, pain medication usage, severity and frequency of headaches, abdominal pain, constipation and/or diarrhea, number of bowel movements per day, and general energy level, commencing 1 week before the initiation of treatment. Parents or guardians assisted with the completion of the diaries if necessary. For 18 of 24 subjects the diaries were electronic, with data entered on hand-held personal dig-

**TABLE 2 Exploratory Efficacy Evaluation in the 24 Pediatric Patients With FD**

Disease Manifestation	Outcome Measure	Assessment
Metabolic	<i>Gb</i> <sub>3</sub>	Plasma and urine sediment
Cardiac	LVM	Echocardiography
Renal	eGFR proteinuria albuminuria	Counahan-Barratt equation urine collection
Autonomic neuropathy	Heart rate variability	2-h ambulatory monitor
Sensory neuropathy	Neuropathic pain	BPI
Hypohidrosis <sup>a</sup>	Sweating	QSART
Morbidity	Fabry symptomatology QoL	Diaries HUI

<sup>a</sup> Conducted at National Institutes of Health only.

ital assistants, with transmission of the data to a central database at 8:00 PM each evening. For the remaining 6 subjects entries were recorded in article diaries, which were returned to the investigator's site every 2 weeks.

#### QoL

QoL was measured by the use of the Health Utility Index (HUI).

#### Quantitative Sudomotor Axon Reflex Test

Sweating in FD was assessed quantitatively by quantitative sudomotor axon reflex test (QSART).<sup>18,25</sup>

#### Repeated Measurements

The echocardiogram and 2-hour ambulatory monitoring were performed again after the final dose of agalsidase alfa (week 25 or 26). Other measurements were repeated at weeks 9 and 17 and after the final dose.

#### Statistics

Methods of descriptive statistics were applied, mainly as frequency analysis. Changes over time in variables, such as eGFR, proteinuria (or microalbuminuria), plasma and urine sediment Gb<sub>3</sub>, heart rate variability, and brief pain inventory (BPI) were analyzed using the appropriate comparative statistical test, that is, paired *t* test for parametric data or the Wilcoxon signed-rank test for ordinal data. All of the calculations were performed with SAS version 8.2 software (SAS Institute, Cary, NC). Values are expressed as mean  $\pm$  SEM for normally distributed data and as median and full range for nonparametric data. The differences in pain medication were assessed by defining a contingency table with 2 variables, dose A (initial dose) and dose B (reduced dose). Differences were compared with Fisher's exact test.

## RESULTS

### Subjects

Twenty-four pediatric patients (19 boys and 5 girls) between 6.5 and 18 years of age with a confirmed diagnosis of FD were enrolled in the study. Mean age of the boys was 11.5 years (range: 6.5–18 years) and that of the girls was 13.5 years (range: 8–17 years). Baseline demographic characteristics of these patients are presented in Table 1. All 24 patients completed the study having received all 13 biweekly agalsidase alfa infusions.

### Safety

#### Infusion Reactions

One or more infusion reactions were reported in 7 (29%) of the 24 patients. Six of 7 individuals with infusion reactions were boys. The reactions typically consisted of rigors, flushing, nausea, and pyrexia, with or without headache, but none was considered to be severe in intensity. Three patients had 1 infusion reaction, 3

subjects had 3 infusion reactions, and 1 patient experienced 5 infusion reactions. Overall, infusion reactions occurred with only 17 (5.4%) of 312 infusions. In only 1 case was an infusion stopped prematurely and not restarted. The mean onset of infusion reactions was at week 9 (range: 3–19 weeks). The reactions were prevented by premedication with antihistamines and/or steroids or by lengthening the infusion duration for subsequent infusions. Five patients required premedication for infusion reactions.

#### Antiagalsidase Alfa Antibodies

One 17-year-old boy tested transiently positive for immunoglobulin (Ig) G antiagalsidase alfa antibodies at week 9 but was negative when tested subsequently at weeks 17 and 26. No IgE antiagalsidase alfa antibodies were detected in any patient during the 26-week study period.

#### Adverse Events

Twenty-three subjects experienced  $\geq 1$  adverse event during this study. Most were mild to moderate in severity, were considered by the investigator not to be related to agalsidase alfa, and were events that would be expected to occur in healthy children or children with FD. During the study period, no patient was withdrawn from the study because of an adverse event, and no deaths occurred. A total of 4 serious adverse events were reported and included a cerebrovascular accident, hospitalization for a neuropathic pain crisis, hospitalization for evaluation of hematuria and proteinuria, and hospitalization for abdominal pain. All 4 were considered by investigator to be unrelated to agalsidase alfa treatment. One 16-year-old patient had repeated small-vessel strokes, commencing at 14 years of age, which continued on enzyme replacement (Fig 1). This patient had an intensive workup that has not uncovered a prothrombotic disorder other than FD. However, an unrecognized susceptibility factor for strokes, in addition to FD, cannot be excluded, because his presentation with recurrent small-vessel strokes at this early age is unusual.

### Exploratory Efficacy Assessments

#### Kidney Function

Mean baseline eGFR was  $121 \pm 5.0$  mL/min per 1.73 m<sup>2</sup> for the total study population of 24 Fabry children. This

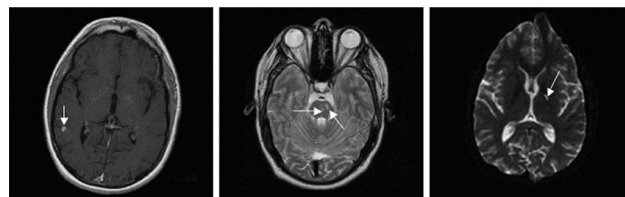
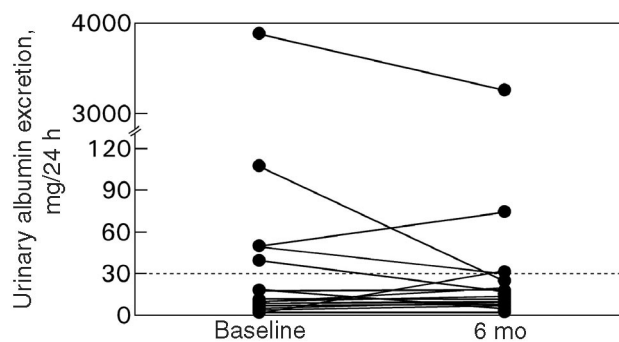


FIGURE 1

Small-vessel disease and strokes in a 16-year-old male patient with FD, documented by MRI (T1, T2, and fluid-attenuated inversion recovery). Arrows indicate sites of lesions.

normal mean eGFR did not change significantly during the 26 weeks of agalsidase alfa treatment. After 9 weeks it was  $119.2 \pm 3.6$  mL/min per  $1.73 \text{ m}^2$ , after 17 weeks,  $120.0 \pm 3.9$  mL/min per  $1.73 \text{ m}^2$ , and at the end of the study (after 26 weeks),  $116.0 \pm 3.9$  mL/min per  $1.73 \text{ m}^2$ . At baseline, 15 subjects had normal eGFR (eGFR  $> 90$  and  $\leq 135$  mL/min per  $1.73 \text{ m}^2$ ), and 7 patients had an eGFR  $> 135$  mL/min per  $1.73 \text{ m}^2$ . Only 2 subjects showed evidence of kidney dysfunction at baseline (National Kidney Foundation chronic kidney disease stage 2 as defined in eGFR between 60 and 89 mL/min per  $1.73 \text{ m}^2$ ).<sup>38</sup> Overall, renal function remained normal after 6 months of ERT. Interestingly, the eGFR of patients with possible hyperfiltration came back to the reference range. The effects of 6-month infusions with agalsidase alfa in each of these chronic kidney disease subgroups are presented graphically in Fig 2.

Proteinuria was evaluated in 21 Fabry children at both baseline and after 25 weeks of treatment. In the 16 patients without proteinuria or microalbuminuria at baseline (urine albumin  $< 30$  mg/24 hours), 15 remained normal during the study (8.6 mg/24 hours at baseline (range: 1.7–19.2 mg/24 hours) and 8.9 mg/24 hours (range: 2.4–31.2 mg/24 hours) after 6 months (Fig 3). In the 4 children with microalbuminuria at baseline (urinary albumin: 30–300 mg/24 hours), median albumin excretion decreased from 50 mg/24 hours (range: 39.7–108) mg/24 hours at baseline to 27.6 mg/24 hours (range: 15.9–74.9 mg/24 hours; *n* of subgroup is too small for formal statistics). The patient with gross proteinuria at baseline demonstrated a modest decrease in her proteinuria from 3876 to 3262 mg/24 hours. This patient was found to have an IgA nephropathy and received treatment with steroids and an angiotensin-converting enzyme inhibitor. No other patients



**FIGURE 3**  
Effects of agalsidase alfa on proteinuria and microalbuminuria in pediatric patients with FD. —, upper limit of normal (30 mg/24 hours), and 30 to 300 mg per 24 hours is considered microalbuminuria.

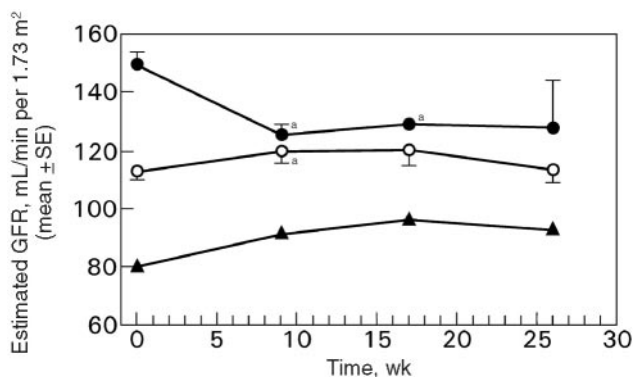
received angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.

#### Plasma Gb<sub>3</sub>

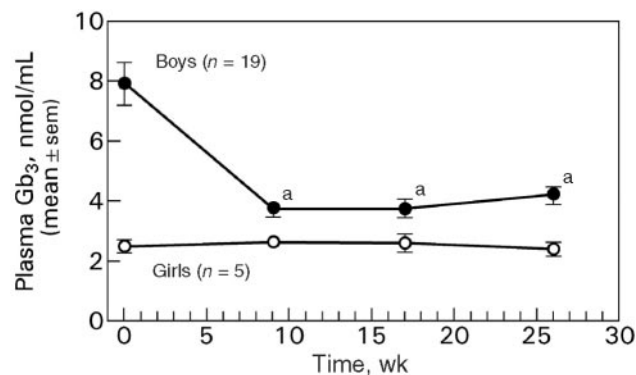
Mean baseline plasma Gb<sub>3</sub> levels and the effect of 26 weeks of agalsidase alfa on plasma Gb<sub>3</sub> levels are shown in Fig 4. Mean baseline fasting plasma Gb<sub>3</sub> was above normal at  $7.91 \pm 0.71$  nmol/mL for the 19 boys and was normal ( $< 3.0$  nmol/mL) at  $2.54 \pm 0.25$  nmol/mL for the 5 girls. Among the 19 boys, mean plasma Gb<sub>3</sub> was significantly reduced by agalsidase alfa therapy at all of the time points measured ( $P < .001$ ; Fig 4). In contrast, plasma Gb<sub>3</sub> levels among the 5 girls, which were at normal levels at baseline, did not change.

#### Urine Sediment Gb<sub>3</sub>

As was the case with plasma Gb<sub>3</sub>, baseline urine sediment Gb<sub>3</sub> levels in boys with FD were higher than in girls with the disease ( $1929 \pm 578$  vs  $141 \pm 60$  nmol/g of creatinine, respectively). After 25 weeks of treatment with agalsidase alfa, mean urine sediment Gb<sub>3</sub> levels decreased to  $957 \pm 344$  nmol/g of creatinine among the 19 boys ( $P = .096$ ) and to  $88 \pm 43$  nmol/g of creatinine in girls ( $P = .125$ ). Although 6 of 19 boys demonstrated



**FIGURE 2**  
Kidney function in 24 pediatric patients with FD by baseline chronic kidney disease stages. ●, Patients with FD with eGFR  $> 135$  mL/min per  $1.73 \text{ m}^2$  ( $n = 7$ ); ○, patients with normal renal function (National Kidney Foundation stage 1) with baseline eGFR 90 to 135 mL/min per  $1.73 \text{ m}^2$  ( $n = 15$ ); ▲, patients with evidence of renal dysfunction (chronic kidney disease stage 2) at baseline with eGFR 60 to 89 mL/min per  $1.73 \text{ m}^2$  ( $n = 2$ ). eGFR was estimated in pediatric patients using the Counahan-Barratt equation.<sup>37</sup> <sup>a</sup> $P < .05$  versus baseline (time 0; Wilcoxon signed-rank test).



**FIGURE 4**  
Effects of agalsidase alfa plasma Gb<sub>3</sub> in pediatric patients with FD stratified by gender. <sup>a</sup> $P < .001$  versus baseline (time = 0).

an unexpected increase in urine sediment Gb<sub>3</sub>, the overall median change from baseline for the 19 boys was still a decrease of 78%.

#### Cardiac Structure, Function, and Heart Rate Variability

Left ventricular wall thicknesses and cavity diameters were measured at baseline and after 25 weeks of agalsidase alfa therapy using standard two-dimensional and M-mode echocardiography. All 24 of the children had left ventricular mass (LVM) indexed for height (LVM/h) that was within the reference range at baseline (upper limit of normal for boys = 51 g/m<sup>2.7</sup> and for girls = 48 g/m<sup>2.7</sup>).<sup>39,40</sup> The mean baseline LVM/h for the 19 boys was 32.4 ± 1.3 g/m<sup>2.7</sup> and 36.0 ± 4.0 g/m<sup>2.7</sup> for the 5 girls. After 25 weeks of treatment, mean LVM/h showed a nonsignificant decrease in both boys and girls to 31.4 ± 1.4 g/m<sup>2.7</sup> and 32.8 ± 2.3 g/m<sup>2.7</sup>, respectively. However, it was noteworthy that the 3 children with high-normal LVM/h at baseline (>40 g/m<sup>2.7</sup>, 2 girls and 1 boy) demonstrated a 15% mean decrease in LVM/h after 25 weeks of agalsidase alfa therapy. Mean ejection fraction was normal at baseline and remained normal after 25 weeks of treatment.

#### Heart Rate Variability

Analyses of heart rate variability from the 2-hour ambulatory monitor recordings are presented in Table 3. In the 19 boys, after 25 weeks of treatment with agalsidase alfa, mean RR interval increased slightly, reflecting a small decrease in mean heart rate. In contrast, the mean values of all indices of heart rate variability improved (increased) in boys compared with their baseline values. The SDNN increased significantly from 75.5 ± 5.3 milliseconds at baseline to 95.9 ± 7.8 milliseconds after 25 weeks of agalsidase alfa therapy (*P* < .02). Similarly, the SDNN-i increased from 57.6 ± 4.1 to 69.2 ± 5.1 milliseconds (*P* < .01) and SDANN-i increased from 46.7 ± 3.8 to 65.0 ± 6.3 milliseconds (*P* < .01). r-MSSD also increased from 32.9 ± 4.1 to 45.7 ± 4.4 milliseconds (*P* < .01), and pNN50 also increased from 10.8% ± 2.5% to 18.3% ± 2.8% (*P* < .01). The means of these

heart rate variability parameters among the 5 girls were within normal limits at baseline, and whereas the means fell slightly after 25 weeks of treatment, these mean values remained within normal limits.

#### Neuropathic Pain, Concomitant Medication, and QoL

In boys, the mean BPI "pain at its worst" score (0 = none, 10 = most severe) decreased from 6.06 ± 0.80 at baseline to 4.42 ± 0.74 after 25 weeks of agalsidase alfa treatment. A decrease in BPI pain at its worst was also observed in the 5 girls, who had started with a slightly lower mean pain score at baseline (5.67 ± 1.45), which then decreased to 4.75 ± 1.03 after 25 weeks of agalsidase alfa therapy. These changes were not statistically significant. Eleven patients were on anticonvulsants (Tegretol or gabapentin) for neuropathic pain. Six (55%) of these 11 patients were able to reduce or stop the use of neuropathic pain medications (*P* = .012). The other 5 remained on constant doses. Twenty-one patients were on nonsteroidal anti-inflammatory drugs on a regular or occasional basis during the study. One patient received sertraline for obsessive-compulsive disorder; 1 patient methylphenidate for attention-deficit/hyperactivity disorder, 1 patient citalopram for depression, and 1 patient divalproex and 1 patient sumatriptan for headache. Pancrelipase, pancreatin, and perenterol were given for gastrointestinal complaints, for example, recurrent diarrhea and abdominal pain, in 1 patient each.

The majority of these pediatric patients had normal or near normal QoL at baseline as judged by HUI3 or HUI2 questionnaires, and remained stable during this 26-week study.

#### Sweat Measurements

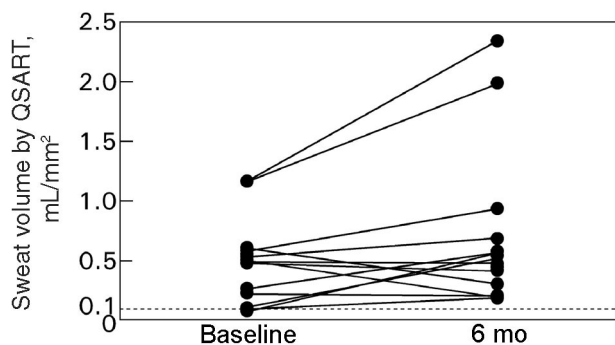
QSART sweat testing was performed on 13 children with FD (12 boys and 1 girl) at the National Institutes of Health, because the equipment was only available at this study site. At baseline, 3 subjects had anhidrosis (as defined by a sweat volume of <0.1 μL/mm<sup>2</sup>). After 25 weeks of agalsidase alfa therapy, all 3 of the patients with anhidrosis had measurable sweating (Fig 5). For the

**TABLE 3 Heart Rate Variability Parameters From 2-Hour Ambulatory Recordings in 23 Pediatric Fabry Patients at Baseline and After 25 Weeks of Agalsidase Alfa Therapy**

Variable	Boys (n = 18)		Girls (n = 5)	
	Baseline	25 wk	Baseline	25 wk
Mean RR, ms	627 ± 19	641 ± 26	794 ± 90	675 ± 53
SDNN, ms	75.5 ± 5.3	95.9 ± 7.8 <sup>a</sup>	138.6 ± 23.1	102.6 ± 8.3
SDNN-i, ms	57.6 ± 4.1	69.2 ± 5.1 <sup>b</sup>	105.2 ± 18.8	85.6 ± 9.0
SDANN-i, ms	46.7 ± 3.8	65.0 ± 6.3 <sup>b</sup>	78.5 ± 23.3	52.8 ± 4.9
r-MSSD, ms	32.9 ± 4.1	45.7 ± 4.4 <sup>b</sup>	91.9 ± 21.3	72.7 ± 17.9
pNN50, %	10.8 ± 2.5	18.3 ± 2.8 <sup>b</sup>	39.9 ± 8.7	28.3 ± 7.1

<sup>a</sup> *P* < .02;

<sup>b</sup> *P* < .01, Wilcoxon signed-rank test.



**FIGURE 5** Effects of agalsidase alfa on sweating as assessed by QSART.

overall group, the mean sweat volumes changed from  $0.48 \pm 0.36 \mu\text{L}/\text{mm}^2$  to  $0.73 \pm 0.68 \mu\text{L}/\text{mm}^2$  ( $P = .06$ , paired 2-tailed  $t$  test).

## DISCUSSION

The present 6-month, open-label study was a prospective trial to assess safety and to explore efficacy of ERT in pediatric Fabry subjects ages 6.5 to 18 years. The results of the study showed that treatment with agalsidase alfa at a dose of 0.2 mg/kg delivered as a 40-minute intravenous infusion every other week for 25 weeks is safe and well tolerated in these young patients. The safety observations made in the clinical trial in children and adolescents with FD are consistent with the clinical experience with agalsidase alfa in adult patients with FD.<sup>23,26,29</sup> Based on the safety data, we do not recommend the general use of premedication unless an infusion reaction occurs. The sustained decrease in plasma Gb<sub>3</sub> suggests that agalsidase alfa is effective in lowering circulating levels of the substrate.

The involvement of peripheral nerves leads to neuropathic pain and impaired heat and cold sensation and has also been reported to result in autonomic dysfunction.<sup>41-43</sup> ERT with agalsidase alfa has been shown to modestly reduce neuropathic pain and to reduce thresholds for sensing heat and cold.<sup>23,25</sup> We hypothesized that this neuropathic process might also involve the sympathetic and parasympathetic nerves that innervate the heart and thereby result in abnormal heart rate variability. In the present study, mean values of all of the time domain heart rate variability parameters in hemizygous male Fabry children and adolescents were significantly reduced compared with heterozygous female Fabry children and those reported in normal children,<sup>44</sup> and they improved with enzyme replacement. ERT with agalsidase beta has been reported to improve cardiovascular control during orthostatic stress in adult Fabry subjects.<sup>45</sup> The present study in children with the disease demonstrated an initial beneficial response of cardiac autonomic innervation with agalsidase alfa therapy. A cardiovascular autonomic neuropathy has also been described in adults with type 1 and type 2 diabetes and in adolescents with type 1 diabetes.<sup>46,47</sup> In adults with diabetes, decreased heart rate variability is associated with a twofold increase in cardiac mortality, possibly related to sudden death.<sup>47</sup> We previously did not find any difference between adult patients and controls in plasma epinephrine and norepinephrine levels, which suggests a globally intact sympathetic neuronal innervation.<sup>48</sup> It is possible, however, that the parasympathetic innervation is abnormal in this disease. The effect of decreased heart rate variability on mortality in FD remains to be studied.

Left ventricular hypertrophy is commonly observed in adult male and female patients with FD and is thought to contribute to long-term cardiovascular morbidity and

mortality.<sup>49,50</sup> Although the administration of agalsidase alfa was associated with a trend toward a small reduction of LVM in pediatric Fabry boys and girls in the present study, it is important to note that LVM indexed to height in all of these pediatric patients fell within the reference range.

Only 2 (8.3%) of the 24 pediatric patients with FD in the present study showed evidence of mild-to-moderate kidney dysfunction at baseline based on their calculated GFR. However, 4 boys (17%) had microalbuminuria, suggesting that kidney involvement in FD begins at an early age. Microalbuminuria decreased in 3 of 4 subjects with baseline microalbuminuria. Seven patients had an eGFR  $>135 \text{ mL}/\text{min}$  per  $1.73 \text{ m}^2$ . This might either be an early sign of hyperfiltration or an overestimation of the true glomerular filtration rate as suggested in a recent comparative study between iothexol clearance and glomerular filtration rate estimated by the application of the Counahan-Barratt equation.<sup>51</sup>

With regard to neuropathic pain, the improvements in pain assessed by either the BPI or QoL subscores with only 6 months of agalsidase alfa therapy were not statistically significant. However, 55% of patients who were on anticonvulsive medication for neuropathic pain were able to decrease or cease their consumption of these drugs. These changes in severity of neuropathic pain and in QoL in this open-label trial might be confounded by a placebo effect or improved care.

Three anhidrotic patients developed sweating as determined by QSART. The final QSART determination was performed 14 days after the last infusion of agalsidase alfa. Previous observations have shown that the improvement in sweat function may last  $\sim 7$  to 10 days after dosing. The sweat test results obtained in the present study may underestimate the ability of ERT to improve sweat function in pediatric patients.<sup>25</sup>

The results of the present pediatric open-label, prospective clinical trial extend the observations in adults and confirm that agalsidase alfa is safe and shows some effectiveness in children and adolescents with FD. Because ERT in adult patients with advanced FD has limited efficacy,<sup>26,33</sup> it is reasonable to hypothesize that ERT, if started at an early age, may slow or even prevent the development of irreversible changes in the heart, kidney, and cerebrovascular system. We regard FD as a risk factor for common complications including stroke. We have shown recently that the vascular risk is further modulated by other genetic factors.<sup>52</sup> These conclusions are illustrated by the continuation of strokes in the 16-year-old male patient while on enzyme replacement. Strokes continued to occur in this patient after  $>18$  months of ERT. This observation is consistent with ongoing or new-onset stroke in adults who receive agalsidase alfa or beta observed by us and others.<sup>29-33</sup> Taken together, they emphasize the preventive goal of this therapy.

The effect of antibodies on Gb<sub>3</sub> clearance has been described before and deserves careful follow-up with regard to its long-term effect on clinical efficacy.<sup>26,53</sup> Although the results presented here demonstrate that bi-weekly infusions of agalsidase alfa in pediatric patients with FD are safe, it is not known whether the otherwise encouraging preliminary results of these multiple exploratory efficacy assessments will translate into long-term clinical benefit. Future clinical trials and/or thorough and systematic long-term observations of clinical outcomes in patients receiving ERT are needed to document the long-term corrective, and especially preventive, results of ERT with agalsidase alfa in pediatric patients with FD. It should be possible to assess the potentially preventive effects of ERT in a prospective study over several years by randomly assigning patients to early versus delayed ERT.

### Study Limitations

Because this study was designed mainly to assess safety, its main drawback is the lack of a placebo-controlled group. The commercial availability of ERT in the United States and in Europe made it difficult to conduct a long-term, double-blind, placebo-controlled study in Fabry children. Although this study is, at the moment, the largest trial of ERT in pediatric patients with FD, the study duration of only 6 months limits the ability to draw conclusions regarding longer-term therapy. The patients were not selected for specific signs or symptoms and were often normal or near normal. Therefore, the number of patients in various subgroups was too small to conduct statistical analyses, such as for the changes in albuminuria or LVM in the abnormal subjects.

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## Enzyme-Replacement Therapy With Agalsidase Alfa in Children With Fabry Disease

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