

# Elevated Cerebral Blood Flow Velocities in Fabry Disease With Reversal After Enzyme Replacement

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**Background and Purpose**—Fabry disease is an X-linked inherited disorder resulting from a deficiency of  $\alpha$ -galactosidase A. Cerebrovascular disease in Fabry disease includes small-vessel disease and larger-vessel ectasia in a predominantly posterior distribution. We assessed transcranial Doppler (TCD) blood flow velocities in naive and enzyme-treated Fabry patients.

**Methods**—TCD was used to noninvasively examine patients with Fabry disease for abnormal cerebral blood flow velocities. TCD measurements were also made during CO<sub>2</sub> retention by breathholding to examine cerebrovascular vessel reactivity. Twenty-six patients were enrolled in a 6-month, double-blind, placebo-controlled trial of enzyme replacement therapy consisting of biweekly intravenous  $\alpha$ -galactosidase A infusions, with a subsequent 18-month follow-up in an open-label trial. Statistical analysis consisted of applying a mixed-effects ANOVA model for correlated outcomes.

**Results**—Peak velocity, mean velocity, pulsatility index, and resistance index were found to be significantly higher in patients compared with control subjects. When the individual vessels were considered, elevated flow velocities were found in the middle cerebral M1 branch and the posterior cerebral artery. Enzyme replacement therapy significantly decreased peak, mean, and end-diastolic velocities and flow acceleration at the 18-month follow-up time point.

**Conclusions**—Patients with Fabry disease have elevated cerebral blood flow velocities. These velocities significantly improved with enzyme replacement therapy. (*Stroke*. 2002;33:525-531.)

**Key Words:** blood flow velocity ■ cerebrovascular accident ■ cerebrovascular disorders ■ Fabry disease ■ ultrasonography, Doppler, transcranial

Fabry disease is an X-linked recessive disorder caused by a deficiency of  $\alpha$ -galactosidase A, a lysosomal hydrolase required to cleave the terminal  $\alpha$ -galactosyl moiety from globotriaosylceramide and other glycoconjugates.<sup>1</sup> Deficiency of  $\alpha$ -galactosidase A leads to accumulation of metabolic intermediates, particularly globotriaosylceramide (Gb<sub>3</sub>), resulting in the systemic manifestations of the disease. Onset usually occurs in childhood with pain crises and acroparesthesia, hypohidrosis, angiokeratoma, and corneal and lenticular abnormalities. Severe morbidity follows cardiac, renal, and cerebrovascular involvement and tends to occur in the fourth decade.<sup>2-6</sup> Accumulation of Gb<sub>3</sub> occurs particularly in vascular smooth muscle and endothelial cells with associated arterial/arteriolar endothelial cell dysfunction and development of tissue ischemia and infarction.

Mitsias and Levine<sup>7</sup> reviewed cerebrovascular involvement in 42 hemizygous Fabry patients and found a predominance of vertebrobasilar or posterior circulation disease with relatively infrequent events occurring in the anterior circulation.

Dolichoectasia of the posterior cerebral circulation arteries (dilated arteriopathy) and intimal thickening of small and medium-sized arteries were also noted. Crutchfield et al<sup>8</sup> detected an increased burden of small-vessel disease with age in a longitudinal MRI study of 50 Fabry patients. In another study, Moore et al<sup>9</sup> also found a selective abnormality in the posterior circulation using [O<sup>15</sup>]H<sub>2</sub>O and PET. Although all these studies suggest a posterior predominance of disease, a significant burden of anterior circulatory disease was also demonstrated.<sup>9</sup> The mechanism by which local cerebrovascular involvement translates into early onset and increased risk of stroke in these patients is unclear. Transcranial Doppler (TCD) is a standard, noninvasive bedside technique used to assess cerebral blood flow velocity in intracranial vessels. TCD has been used in many central nervous system disorders to allow insight into cerebrovascular function.<sup>10,11</sup>

Normative data exist for the mean flow in the circle of Willis vessels<sup>12-15</sup> and for CO<sub>2</sub> reactivity.<sup>16-18</sup> It is not known

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how device dependent these data may be. Data on flow acceleration (FA) are limited, with the study by Kelley et al<sup>19</sup> representing one of the definitive reports. Recently, enzyme replacement therapy (ERT) in a single-dose phase I trial was shown to be safe and to reduce the hepatic burden of Gb<sub>3</sub>, whereas a randomized, double-blind, placebo-controlled trial showed a significant decrease in neuropathic pain with stabilization or improvement in renal function and a reduction in cardiac ECG abnormalities.<sup>20,21</sup> We undertook TCD analysis of patients with Fabry disease to evaluate flow velocity and CO<sub>2</sub> reactivity compared with an age-matched control population. Subsequently, TCD was used to examine treatment efficacy after  $\alpha$ -galactosidase A ERT in a randomized, double-blind, placebo-controlled trial and in an 18-month open-label follow-up study.

### Patients and Methods

Sixty-three hemizygous male Fabry patients (age, 19 to 56 years), together with 31 male control volunteers (age, 26 to 49 years), were enrolled in accordance with institutional guidelines. The Institutional Review Board of the National Institute of Neurological Disorders and Stroke approved the study; all healthy subjects and patients gave written, informed consent. Subjects were examined through the right and left temporal windows in a sitting position except for the ophthalmic artery (OA), which was insonated transorbitally, and the extracranial internal carotid artery, which was insonated by a submandibular approach. Transcranial examination was restricted to the intracranial internal carotid artery [TICA(C1)], the M1 and M2 segments of the middle cerebral artery [MCA(M1), MCA(M2)], and the A1 segment of the anterior cerebral artery [ACA(A1)] in the anterior circulation. In the posterior circulation, the P1 or P2 segment of the posterior cerebral artery [PCA(P1), PCA(P2)] was examined. TCD waveforms were obtained with a 2.5-MHz probe by use of a 500M Neurovision TCD instrument (Multigon Industries Inc). Vessel discrimination was determined by insonation direction and depth as follows: TICA(C1), 6.4 cm; MCA(M1), 5.0 cm; MCA(M2), 4.0 cm; PCA, 4.0 cm; ACA, 7.0 cm; OA, 5.0 cm; and extracranial internal carotid artery, 2.0 to 2.5 cm. The gate was 11 mm in width. There was a change in angle between the M1 and M2 branches of the MCA. Measurements in the extracranial internal carotid arteries were performed with a 5.0-MHz probe. Replicated Doppler studies obtained in 23 of 63 adult male patients over a 6- to 12-month period were averaged before statistical analysis. All studies were performed bilaterally. Cerebrovascular bed reactivity was examined by insonation of the right and left M1 segments, followed by breathholding for 1 minute. This step involved timed breathholding for 1 minute after a maximal inspiration while the insonation position was held constant over the MCA(M1). The technical difficulty in carrying out these measurements on the MCA(M1) during breathholding precluded similar measurements on the PCA, since even slight movement by the operator resulted in loss of an optimal signal.

The following Doppler parameters were obtained: peak flow velocity (PV), mean flow velocity (MV), end-diastolic velocity (ED), pulsatility index [PI=(PV-ED)/MV], and resistance index [RI=(PV-ED)/PV]. FA was derived from the recorded Doppler waveforms by taking the waveform envelope function and fitting a natural cubic spline function. The envelope function was produced directly from the Multigon 500M device by taking the peak frequency shift in each time bin and converting the value to a velocity measurement. A total of 500 envelope points were output in hexadecimal format with subsequent postprocessing in custom-written C code. Smoothing was performed with a variable-moving average window filter with a width chosen to minimize the total sum of the square difference between the input envelope signal and the output smoothed waveform (convergence). The spline function, differentiated by forward differencing, was initially fitted by 1000 points, and the number of points was increased by a factor of 10 at each subsequent iteration until the convergence criterion of <0.01

m/s<sup>-2</sup> was reached between the *n*th and the (*n*-1)th iteration for the maximum of the derivative function. The FA value was estimated across the whole time sweep of the derivative function. The typical number of pulse cycles per recording was 3; however, this number varied according to the subject's heart rate. Because of noise in the Doppler waveform, not all envelope functions were continuous, and because of singularities, not all FA estimations demonstrated satisfactory convergence. These data were not included in the final statistical analysis of FA. The custom-written C code allowed selection of a partial time bin period if the Doppler waveform was determined to be acceptable for analysis by direct visual inspection.

A total of 26 Fabry patients were selected and randomized in a double-blind, placebo-controlled trial of  $\alpha$ -galactosidase A lasting 6 months. To be included in the trial, patients had to have neuropathic pain related to Fabry disease.  $\alpha$ -Galactosidase A (Transkaryotic Therapies, Inc) was administered every 2 weeks by intravenous infusion; TCD was performed before randomization and after completion of the trial, with another time point and TCD examination 1 year after completion of an open-label continuation ERT trial. Because cardiac output may represent a confounding factor in any TCD examination and secondarily in discriminating any ERT treatment effect, it was estimated from the stroke volume derived from cardiac MRI. Heart rate was determined from the individual TCD waveforms in each patient in the following manner: Each subject's Doppler waveform set was displayed in the order recorded with a graphic user interface written in MATLAB 5.3 (Mathworks Inc) that allowed cursors to be placed at appropriate points in the Doppler waveform. The cursor was placed "by eye" at the initial upstroke of the waveform with the total time period measured for as many discrete pulse cycles as possible (usually 2 to 3) to maximize accuracy of the estimate. Bilateral estimates of heart rate were obtained from the right- and left-hemisphere TCD waveforms. These estimates were then averaged to give a single estimate of the basal heart rate for an individual subject. The product of stroke volume and estimated heart rate gave the derived cardiac output. Cardiac MRI was used to measure left ventricular volume at end systole and end diastole and, by difference, stroke volume. Cardiac MRI was performed before ERT, at 6 months, and at 18 months with calculation of cardiac output at each time point.

### Statistical Analysis

Statistical analysis was performed with SAS using a mixed-effects ANOVA model for correlated outcomes<sup>22</sup> for each Doppler measure (PV, MV, ED, PI, and RI). For a given Doppler parameter, intraindividual correlation among the different vessels was modeled with individual specific random effects, whereas the vessel site and group designation (ERT or non-ERT) were treated as fixed effects. Group level differences were judged significant when group-by-site interactions or group main effects had a value of  $P < 0.01$ . This significance value level reflects a Bonferroni correction accounting for the fact that 5 different vessels were considered. The model was then used to look for statistically significant differences between the Fabry patients and control subjects. The model was also used to examine the differences between the placebo and treatment groups of Fabry patients after completion of the randomized, double-blind, placebo-controlled trial and at the 18-month follow-up time point, at which time the differences in treatment effect were examined by pairwise comparison of the patients' Doppler parameters to their before-treatment values. The null hypothesis for the last comparison indicates that no change would be expected in the Doppler parameters from baseline to follow-up if no treatment effect had occurred after ERT; hence, a significant difference was declared if these differences were significantly different from zero. Subgroup analysis allowed comparisons of individual arteries. Before statistical analysis, data from the right and left hemispheres were averaged for each subject to obtain a more globally representative result and to reduce further the possibility of a type 1 statistical error. The variances of the right and left hemisphere data were not significantly different. The random effect model allows the inherent variability of the cardiovascular system to be accounted for and is therefore more representative of the underlying physiological fluid mechanics.<sup>23</sup>

**Results**

Table 1 and the Figure summarize the mean, SD, and probability value for statistical differences in the hemizygous male Fabry patients compared with the control group for the Doppler parameters (PV, MV, ED, PI, RI) averaged across the right and left hemispheric vessels. Comparison of the control and Fabry groups showed a significantly increased class effect (group or group-vessel interaction term) for all Doppler parameters (PV,  $P=0.0001$ ; MV,  $P=0.0002$ ; ED,  $P=0.041$ ; PI,  $P=0.026$ ; RI,  $P=0.0001$ ). No significant effect was found in the difference in the M1 Doppler parameters after breathholding for 1 minute. The absolute values of PV (control group, 95.71cm/s; patient group, 110.20 cm/s;  $P=0.0420$ ) and PI (control group, 0.741; patient group, 0.843;  $P=0.0139$ ) were significantly different.

At the 6-month time point, no significant treatment effect was found between the ERT and placebo groups for any of the Doppler parameters (PV, MV, ED, PI, RI, FA). Cardiac output also showed no significant difference between groups (ERT group,  $6.13\pm0.99$  L/min; placebo group,  $6.70\pm1.33$  L/min), with a normal resting cardiac output in the range of 4 to 8 L/min. The estimated stroke volume for the ERT group was  $100\pm15$  mL and that for the placebo group was  $94\pm11$  mL; the difference was not significant.

After completing the initial trial, all patients were enrolled in a 1-year open-label continuation trial of ERT. A further follow-up TCD study was performed when all patients had completed either 12 or 18 months of ERT. The baseline time point (before ERT) TCD parameter values were compared with the 18-month follow-up values in a pairwise fashion and analyzed as previously described. The overall class differences for the PV, MV, and ED parameters were significantly different from baseline at the 18-month follow-up ( $P=0.0001$ , 0.0001, and 0.0007, respectively), indicating a significant treatment effect. FA nearly reached statistical significance after Bonferroni correction ( $P=0.0123$ ). Individual vessel differences are presented in Table 2. As can be seen from Table 2, MCA(M1) and MCA(M2) were found to be significantly different from baseline in terms of PV, MV, and ED. Doppler velocities in these vessels decreased toward normal. The FA value also significantly decreased in the ACA and MCA(M2). After breathholding, a trend reduction in all 3 Doppler velocity parameters toward normal was noted at the 18-month time point with PV=82.53 and 100.42 cm/s ( $P=0.077$ ), MV=52.87 and 65.77 cm/s ( $P=0.0575$ ), and ED=39.13 and 49.63 cm/s ( $P=0.0517$ ), respectively. There was no significant difference in either heart rate or cardiac output between baseline and the 18-month follow-up.

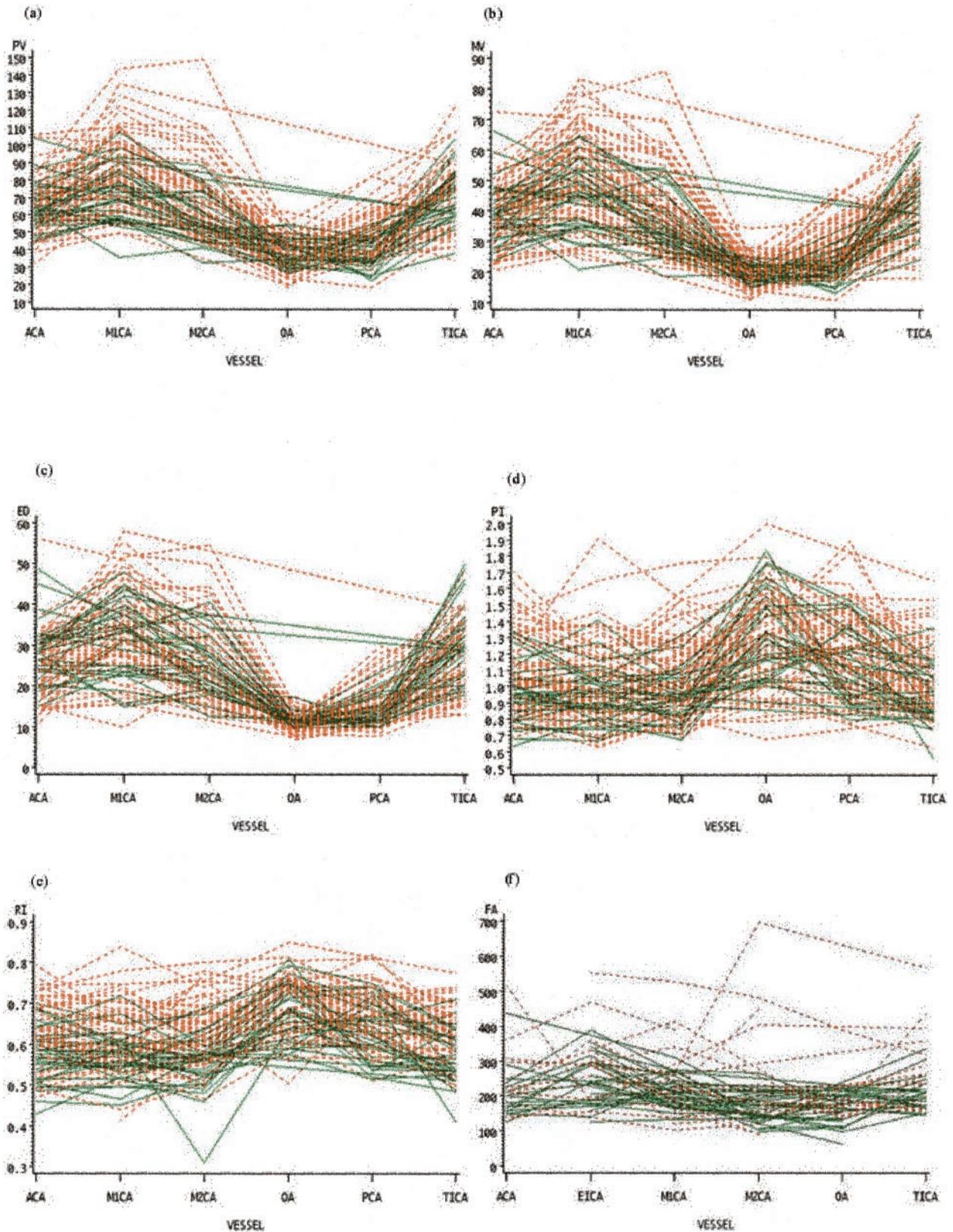
**Discussion**

The results presented here demonstrate a global elevation in the Doppler blood velocity parameters in patients with Fabry disease. This should be considered in relation to the specific vessel subgroup analysis in which only the MCA(M1) and PCA achieved significance. These vessels represent the dominant supply of the cerebral circulation and hence might account for the significance of the global PV, MV, and ED Doppler parameters despite a lack of subgroup significance in the other vessels examined, ie, the TICA, ACA, and

**TABLE 1. Doppler Flow Velocity Parameters PV, MV, ED, PI, RI, and Vessel Wall Compliance FA Index for Male Hemizygotes and Control Subjects**

	Anterior Circulation		
	Hemizygotes	Control Subjects	<i>P</i>
<b>TICA</b>			
PV, cm/s	73.18±18.63	72.20±14.37	0.7996
MV, cm/s	42.51±11.42	44.24±9.84	0.4769
ED, cm/s	26.24±8.38	31.01±8.55	0.0168
PI	1.10±0.22	0.94±0.17	<u>0.0012</u>
RI	0.64±0.06	0.56±0.06	<u>0.0002</u>
FA, cm/s <sup>2</sup>	267.67±131.71	201.62±47.20	<u>0.0069</u>
<b>ACA</b>			
PV, cm/s	62.82±16.68	69.26±12.97	0.4642
MV, cm/s	36.93±10.90	39.65±9.21	0.2358
ED, cm/s	23.10±8.30	27.92±7.65	0.0121
PI	1.12±0.22	0.95±0.18	<u>0.0007</u>
RI	0.64±0.07	0.57±0.06	<u>0.0001</u>
FA, cm/s <sup>2</sup>	239.06±115.04	208.29±76.02	0.3221
<b>MCA (M1)</b>			
PV, cm/s	88.25±21.96	73.47±16.39	<u>0.0006</u>
MV, cm/s	53.32±13.18	45.13±11.00	<u>0.0043</u>
ED, cm/s	33.81±10.76	31.77±8.99	0.3452
PI	1.06±0.24	0.94±0.16	0.0123
RI	0.62±0.08	0.56±0.05	<u>0.0005</u>
FA, cm/s <sup>2</sup>	231.15±115.44	211.33±54.54	0.1715
<b>MCA (M2)</b>			
PV, cm/s	69.82±21.31	58.63±14.05	0.0179
MV, cm/s	40.24±14.47	36.02±9.30	0.1372
ED, cm/s	24.56±10.00	25.42±7.22	0.6713
PI	1.13±0.23	0.94±0.15	<u>0.0003</u>
RI	0.65±0.07	0.55±0.07	<u>0.0001</u>
FA, cm/s <sup>2</sup>	291.13±181.17	166.57±52.57	0.0002
<b>MCA (M1) BH</b>			
PV, cm/s	110.20±29.4	95.71±26.29	NA
MV, cm/s	68.91±19.82	64.30±19.05	NA
ED, cm/s	52.17±16.77	49.53±15.81	NA
PI	0.87±0.28	0.74±0.11	NA
RI	0.52±0.08	0.49±0.05	NA
<b>Posterior Circulation</b>			
<b>PCA</b>			
PV, cm/s	46.15±11.92	37.03±8.22	<u>0.0003</u>
MV, cm/s	25.33±7.78	20.40±3.68	<u>0.0014</u>
ED, cm/s	14.78±5.20	13.07±2.34	0.0758
PI	1.29±0.26	1.14±0.22	0.1002
RI	0.67±0.07	0.63±0.07	0.0320

BH indicates breathhold. Probability values compare the difference in Doppler parameters for the vessel and group means between the Fabry cohort and the control group using mixed-effects ANOVA for correlated measures.  $\chi^2$  Tests were used to obtain probability values. A value of  $P<0.01$  was regarded as significant after applying a further Bonferroni correction to the derived probability value. Significant *P* value is underlined. None of the parameters measured was significantly different in the OA.



Composite diagram illustrating the scatter of the Fabry and control groups against each measured vessel. Solid green line shows the control group; dashed red line, Fabry patient group. a, Plots of PV (a; cm/s), MV (b; cm/s), ED (c; cm/s), PI (d); RI (e); and FA (f; cm/s<sup>2</sup>). EICA indicates extracranial internal carotid artery; M1CA, MCA(M1); and M2CA, MCA(M2).

**TABLE 2. Estimates and Probability Values for Comparison of the Difference in the Doppler Parameter Vessel Group Means of the ERT Fabry Cohort at Baseline and After 18 Months of Follow-Up Using the Mixed-Effects ANOVA Model for Correlated Measures**

	TICA	ACA	MCA (M1)	MCA (M2)	PCA	ECICA
PV, cm/s	-4.342	-2.760	-18.93	-22.81	-2.733	-2.740
<i>P</i>	0.1978	0.4458	<u>0.0002</u>	<u>0.0001</u>	0.4973	0.1873
MV, cm/s	-2.412	-1.676	-11.58	-12.97	-1.390	-1.146
<i>P</i>	0.2245	0.4600	<u>0.0004</u>	<u>0.0001</u>	0.4716	0.2534
ED, cm/s	-1.916	-0.835	-7.904	-7.487	-0.406	-0.503
<i>P</i>	0.2379	0.6429	<u>0.0018</u>	<u>0.0010</u>	0.7189	0.6071
FA, cm/s <sup>2</sup>	-71.62	-93.40	-53.72	-131.18	NA	-116.41
<i>P</i>	0.0154	<u>0.0027</u>	0.0110	<u>0.0065</u>	NA	0.0166
PI	-0.002	-0.004	-0.021	-0.083	-0.035	-0.055
<i>P</i>	0.9611	0.9374	0.7064	0.1568	0.8259	0.4365
RI	-0.002	-0.009	-0.009	-0.026	-0.0161	-0.011
<i>P</i>	0.8881	0.6821	0.5899	0.1826	0.4877	0.5880

ECICA indicates extra-cranial internal carotid artery.  $\chi^2$  Tests were used to obtain probability values. A value of  $P < 0.01$  was regarded as significant and is underlined. The estimated decrease in Doppler parameters from baseline is recorded by negative values, with a positive value indicating an increase in that Doppler parameter from baseline. None of the parameters measured was significantly different in the OA.

MCA(M2). This global abnormality improved after ERT as measured by the decrease in PV, MV, ED, and FA. In particular, the decrease in FA may reflect normalization of the vessel wall compliance rather than increased proximal resistance since there was a small relative decrease in PI and RI after ERT. Although this interpretation of the FA findings might be questioned, the alternative—increased proximal stenosis with an associated decreased upstroke of the Doppler velocity waveform—appears unlikely given the normal MR angiography studies in the Fabry group (unpublished observations). It is also entirely possible that the resulting decrease in FA seen after ERT is a combination of a decrease in arterial elasticity/compliance and an alteration in the distal cerebral vessel diameter, resulting in decreased cerebral input resistance.

The study was divided into a number of components. These should be considered separately. Initially, we examined a cohort of hemizygous Fabry patients and compared them directly with an age- and sex-matched control population of clinically healthy subjects. All replicated values for patients were averaged, followed by averaging of the TCD parameters from the right- and left-side insonations. No effort was made to control for potentially confounding variables such as subject anxiety, variation in examiner technique, and time from last meal because such variability would tend to increase the data variance and therefore make a significant result less likely, contrary to the observed findings. The vast majority of patients had normal routine hematological and biochemical laboratory indexes that did not change significantly over time.

The ERT study comprised an initial 6-month randomized, double-blind, placebo-controlled trial in which 14 patients received treatment and 12 patients received placebo. The TCD parameters of the treated and placebo groups were not significantly different as determined by ANOVA of corre-

lated outcomes. This was probably due to insufficient statistical power and the increased variability of TCD because a concurrent study using  $[O^{15}]H_2O$  and PET demonstrated reduced cerebral perfusion after 6 months of treatment in identical patient groups.<sup>24</sup> In contrast, at the 18-month follow-up time point, after conversion of the placebo-controlled study to an open-label trial, a difference was discerned by TCD. At this time point, each patient was used as his own control by comparing the TCD results at 18 months with the values before the beginning of ERT. By 18 months, the ERT effect probably was large enough to be seen by TCD, and combining the initial placebo and treatment groups increased the study power.

The statistical method used, ANOVA for correlated outcomes, together with the further application of a Bonferroni correction, reduced the likelihood of a type 1 error. ANOVA also allowed the comparison of Fabry patients and control subjects for a group effect in terms of the individual dependent variables PV, MV, ED, FA, PI, and RI, with the subsequent subgroup analysis determining the most significantly different vessels. Part of the difficulty in the analysis of TCD data has been how to determine an appropriate global measure of the cerebrovascular system from TCD parameters given the heterogeneity of the end vessel distribution and the statistical correlation of the data. ANOVA for correlated outcomes enables the combined vessel data from 1 patient group to be compared with another. Such analysis has potential applications in other cerebrovascular diseases such as subarachnoid hemorrhage in which accumulated data from multiple vessels could increase the sensitivity of TCD measurements.

No significant alteration occurred in PI or RI after treatment, suggesting limited sensitivity of these indexes as surrogate markers of treatment efficacy in Fabry disease. It is important to consider that the relationship of PI and RI to true

vessel bed resistance is at best qualitative, with a high PI and RI consistent with an increased vascular bed resistance and vice versa. The elevated cerebral blood velocity may result from altered arteriolar endothelial function. Such an interpretation is strengthened by the recent description of an abnormal response in the peripheral forearm vascular bed to intra-arterial acetylcholine in Fabry disease.<sup>25</sup> This altered endothelial function may account for selective involvement of the small perforating cerebral vessels in Fabry disease. The altered response of Fabry vessels to hemodynamic stresses may also contribute to the local procoagulant state, which was previously shown to be present in Fabry disease.<sup>26</sup> Such features may be related to the increased risk of small-vessel disease seen in Fabry disease.

The group differences for all 5 Doppler parameters between the Fabry and control groups support a significant alteration in the cerebral vascular bed hemodynamics in this disease. The significant elevation of cerebral blood flow velocities seen in the MCA(M1) and PCA indicates that both the anterior circulation and posterior circulation are involved. These vessels make up most of the cerebrovascular bed and furthermore account for the origin of most of the deeply penetrating vessels, with the lenticulostriates from the M1 branch and the thalamoperforating arterial group from the PCA. The increased mean cerebral blood flow velocity in Fabry disease could also be secondary to an increased cardiac output. This seems unlikely given the finding of normal cardiac output. Corroboration of the TCD data by a totally different and independent imaging modality using [<sup>15</sup>O]H<sub>2</sub>O and PET also strengthens the above conclusion of elevated cerebral blood flow velocities in Fabry disease.<sup>24</sup> Other organ systems also showed treatment responses to ERT. More specifically, improvement in renal function and cardiac conduction defects and clinical improvements in the severity of neuropathic pain have been found.<sup>21</sup>

Breathholding reactivity assessed by insonation of the MCA(M1) demonstrated a decrease in the absolute PV, MV, and ED Doppler parameters after ERT. However, given the posterior predominance of the cerebrovascular abnormality in Fabry disease, the relevance of measurement on an anterior circulation MCA(M1) vessel may be questioned. The fact that Fabry disease is a diffuse disease may allow some extrapolation from the anterior to the posterior circulation. Additional issues surround the use of breathholding as a technique for examining cerebrovascular reactivity such as induction of a Valsalva response, which will reduce cerebral blood flow. A more appropriate technique for examination of cerebral reactivity would be inhalation of 5% CO<sub>2</sub>. The smaller absolute decrease in PI in Fabry patients with breathholding without a significant change in the difference between before and after breathholding suggests impaired cerebrovascular vasodilation to CO<sub>2</sub>.

The substantially elevated RI in the anterior circulation [TICA, ACA, MCA(M1), OA] but not in the posterior circulation may be the result of the differential innervation in these cerebral regions.<sup>27,28</sup> However, there is no evidence for neurogenic autonomic dysfunction in the forearm by sympathetic microneurography in Fabry patients (Goldstein et al, unpublished results, 1999). This suggests that vascular dys-

function is confined to arteriolar smooth muscle and endothelial cell components. The decreased Doppler blood flow velocities after ERT, together with a more normal response to CO<sub>2</sub> retention, may represent a surrogate maker for a reduced risk of stroke in Fabry disease. However, this has not yet been explicitly tested by such techniques as survival analysis or logistic regression.

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