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# Comparative evaluation of α-galactosidase A infusions for treatment of Fabry disease

*Robert J. Hopkin, MD,[1] John Bissler, MD,[2] and Gregory A. Grabowski, MD[1]*

Enzyme therapy has proven safe and effective in preventing and reversing many manifestations in patients with Gaucher disease. On the basis of this success, enzyme therapy is now becoming a reality for Fabry disease, α-galactosidase A deficiency. Two products, agalsidase alpha and beta, have been tested in pivotal trials. The substantial differences between the study structures and outcome measures have made direct comparisons difficult. Here, the strengths and weaknesses of these trials are compared: achievement of stated endpoints, safety and, potential efficacy. In addition, the need for additional long-term data is emphasized because this is not attainable in short-term trials for a chronic disease. ***Genet Med* 2003:5(3):144–153.**

The remarkable success of enzyme therapy (ET) in Gaucher disease has provided a stimulus for extending this approach to other lysosomal storage diseases.[1] Because of its relative frequency, severity, and debilitating nature, Fabry disease has become a focus of such recent attention.[2,3] Fabry disease has major manifestations in the renal, cardiovascular, and nervous (central, autonomic, and peripheral) systems. The primary etiology of the degenerative effects in these systems relates to abnormal accumulation of globotriaosylceramide [trihexosylceramide (THC)] in the endothelium of circulatory and lymphatic vascular systems. In addition, THC accumulates in cardiomyocytes, mesangial cells, and podocytes of the kidney, neurons of the central and autonomic nervous systems, and cells of the cardiac conduction system. Over 2 to 3 decades, this progressive THC accumulation leads to the major morbid manifestations of Fabry disease by as yet ill-defined mechanisms (Table 1). In affected males, the manifestations begin in childhood, with angiokeratoma and acroparesthesias being the earliest significant manifestations. Although not well documented, isosthenuria and mild proteinuria are probably the first renal abnormalities and occur in the first to second decade. The progression of acroparesthesias and other components of Fabry pain crisis (episodic severe pains) without overt clinical or laboratory abnormalities may lead to misdiagnoses that include psychiatric disorders and rheumatic fever.[4]

In a large study of Fabry hemizygotes, the life expectancy of affected males was decreased by 3.5 decades compared with unaffected, ethnically matched males (Fig. 1). The average age at death in nonrenal transplanted hemizygous males is 41 to 55 years.[5–7] Because the early signs and symptoms can be nonspecific, the diagnosis is often delayed by 10 or more years from the time of initial medical contact.[7] Early diagnosis is essential because many of the manifestations may become poorly reversible or irreversible with age (i.e., the pathologic involvement is significant before becoming clinically apparent so that decreasing glomerular filtration rate (GFR) occurs only after much of the kidney is damaged severely).

Surprisingly for an X-linked disorder, Fabry disease heterozygous females have clinically significant manifestations that lead to a foreshortening of life span by about a decade (Fig. 1).[8] The predominant signs and symptoms of renal, cardiac, and peripheral and central nervous system (CNS) abnormalities are similar in both groups. Notably, renal failure is a much less frequent complication in heterozygote females (Fig. 2). The majority of males have clinically overt renal abnormalities presenting early in the second decade that progress to renal failure by the fourth to fifth decade. Concomitantly, progressive cardiac and CNS abnormalities occur but with less predictable onset or progression. Indeed, our recent analysis of 14 Fabry hemizygotes and 9 heterozygotes indicate an unappreciated high frequency of CNS lesions in the second to third decades as assessed with history, physical examination, and magnetic resonance imaging (MRI).[9] Extensive biochemical, histologic, and clinical reviews of Fabry disease are available that describe the nature of the morbid manifestations.[5–8,10,11] Suffice to say that in the first and second decade, pain, including acroparesthesias and crises of abdominal and other organ pain, predominates. During the second to fourth decade, renal manifestations generally become manifest, and, later, the cardiac and CNS abnormalities appear (Fig. 3). As larger Fabry patient populations are carefully assessed, the richness of the breadth, scope, and diversity of the disease manifestations will become more fully appreciated. Already, the degree and extent of disease manifestations in heterozygous females with Fabry disease are being recognized as much more pervasive than the misnomer, X-linked recessive, implies. The designation of Fabry disease as X-linked or, possibly, X-linked dominant with variable expression in heterozygotes, seems appropriate. Fu-

*From the [1]Division and Program in Human Genetics and [2]Nephrology and Hypertension, Cincinnati Children's Research Foundation, Cincinnati, Ohio.*

*Gregory A. Grabowski, MD, Director, Division and Program in Human Genetics, Children's Hospital Medical Center, TCHRF 1042, 3333 Burnet Avenue, Cincinnati, OH 45229-3039.*

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**Table 1**
Major clinical manifestations of Fabry disease

| Organ system | Manifestation |
|---|---|
| Peripheral nerves | Acroparasthesias |
| Superficial vessels | Angiokeratoma |
| Kidney | Isothenuria, hypertension, renal insufficiency/failure |
| Central nervous system | Strokes, transient ischemic attacks |
| Autonomic nervous system | Hypohydrosis, diarrhea, vasomotor instability |
| Cardiovascular system | Coronary insufficiency, hypertrophic myocardiopathy |

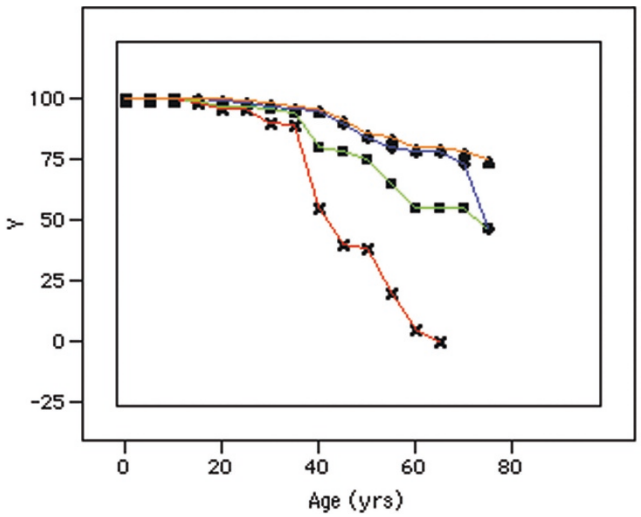


**Fig. 1** Survival of Fabry hemizygotes and heterozygotes (adapted from MacDermot et al.[6] and Desnick[7]). Ethnically matched males with (X, red) and without (□, green) Fabry disease. Similar group of females with (◇, blue) and without (△, orange) Fabry heterozygosity. *y*-axis, percent with phenotype at a specific age.

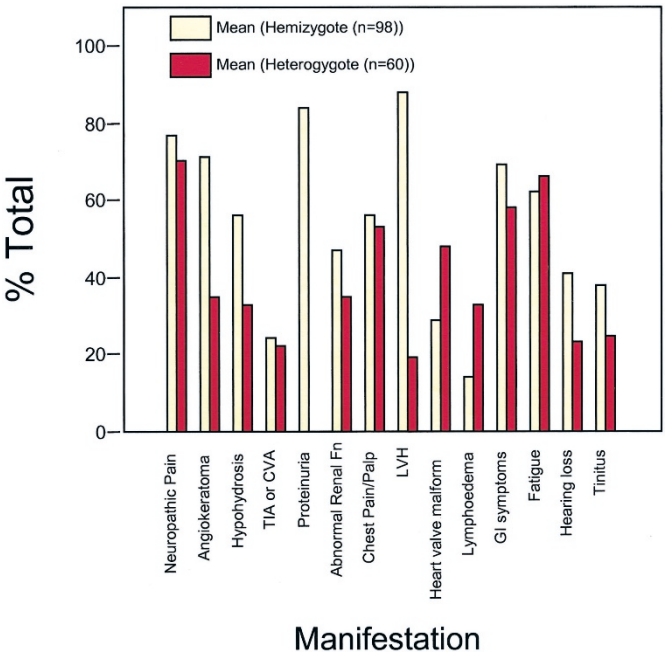


**Fig. 2** Frequency of clinical manifestations among Fabry hemizygotes (beige) and heterozygotes (red). TIA, transient ischemic attack; CVA, cardiovascular accident; LVH, left ventricular hypertrophy.

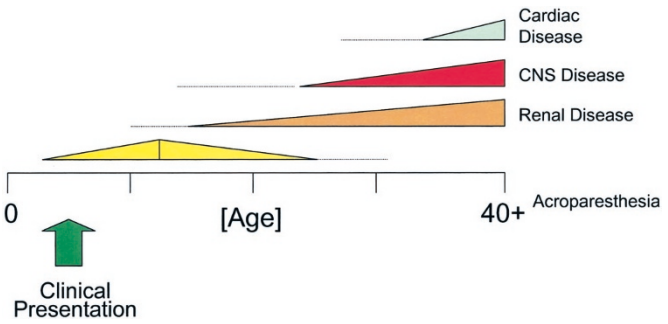


**Fig. 3** Schematic of Fabry disease clinical manifestations for hemizygotes. The renal, cardiac, and central nervous system (CNS) disease are typically promoted by ischemia with transient ischemic attack (TIA), stroke, coronary insufficiency, and progressive renal deterioration. The precise mechanisms of disease initiation or progression are unknown.

ture studies of males and females with Fabry disease should carefully evaluate the frequency and severity of complications.

## MUTATIONS AND α-GALACTOSIDASE A PROPERTIES

Fabry disease results from mutations at the locus for α-galactosidase A that maps to chromosome Xq22. More than 150 mutations have been described in the gene for this lysosomal hydrolase in affected families (Fig. 4).[12–17] Most of these are private mutations encompassing the spectrum of missense, nonsense, and deletion of mutations. Many of these mutations are predicted to lead to the absence of functional enzyme protein, i.e., these patients are cross-reacting immunologic material (CRIM) negative. These patients are generally more severe and are designated as "classic." Some missense mutations lead to residual α-galactosidase A enzymes with significant, albeit low, enzymatic activity (CRIM positive). This difference has clinical and, potentially, therapeutic import. The CRIM positive mutations are important "experiments of nature" that can lead to the variants of Fabry disease that may have specific organ involvement. These patients have progressive myocardial disease and specific mesangial and podocyte abnormalities in the kidney but do not have progressive renal disease leading to failure in the first 5 decades.[8,18–20] The presence of low levels of residual enzyme activity in specific organs or cells apparently delays or prevents some renal manifestations but allows for progressive myocardial disease. Such a "protective" effect on renal function suggests that an appropriate level of enzyme in selected renal cells could delay or prevent the onset or progression of disease in this critical organ. Similarly, renal biopsies of heterozygous females with normal GFR show extensive podocyte THC accumulation, but the vascular endothelium appears spared. Higher enzyme levels may be required to pro-

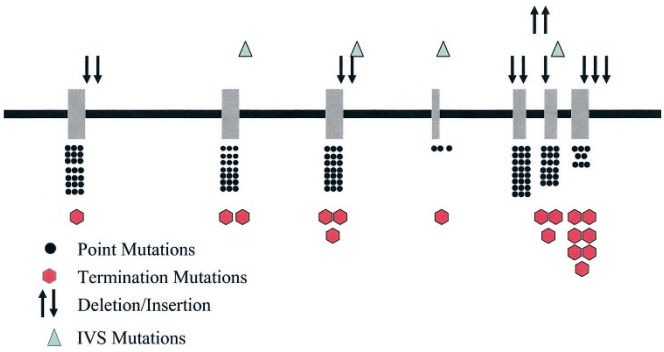


**Fig. 4** Diagram of the mutations in α-galactosidase A found in Fabry disease families. Various types of mutations occur, but none appear to be recurrent. The majority of common mutations result from founder effects in isolated populations. Many of the various mutations lead to an absence of enzyme protein being produced, but residual activity can occur in some of the "milder" variants. IVS, intervening sequence.

tect or reverse the cardiac, particularly the myocardial, lipid accumulation. Studies of tissue chimerism in heterozygous females could provide important insights into the levels of α-galactosidase A necessary for prevention of selected morbid manifestations.

The enzyme, α-galactosidase A, dimerizes into an active protein.[8,21] This glycoprotein has specific N-linked carbohydrate structures attached at residues Asn 108, 161, and 184 whose compositions differ depending on tissue or recombinant source.[22–24] Extensive structural characterization of α-galactosidase A from human tissues is not available, but this has been accomplished with the recombinant enzyme produced in CHO (Fabrazyme) and human fibroblasts (Replagal).[24] In general, for Fabrazyme or Replagal, the oligosaccharide chains on Asn 108 are of the complex type, with fully or partially sialylated terminations at their nonreducing ends. In comparison, the oligosaccharides on Asn 161 and 184 are either oligomannosyl/hybrid or complex in nature. Over 90% of the oligomannosyl/hybrid structures contain M-6-P. The percentage of glycoform that is fully sialylated on Asn 108 is greater for Fabrazyme (approximately 83%) than Replagal (approximately 53%). For Asn 161, similar proportions of the complex oligosaccharides are fully sialylated, as on Asn 108, with 87% and 45% sialylated, respectively. Another difference between Fabrazyme and Replagal at Asn 161 was the greater percentage of oligomannosyl/hybrid (75% vs. 48%) glycoforms and the high degree of M-6-P content (96% vs. 69%) on these structures in Fabrazyme. At Asn 184, content of oligomannosyl/hybrid oligosaccharides also differed from Fabrazyme versus Replagal (96% vs. 66%), and a higher degree of M-6-P was present on these oligosaccharides. Importantly, the CHO produced enzyme, Fabrazyme, is not identical to that used in preclinical trials (see below).

The primary amino acid sequences of α-galactosidase A in Fabrazyme and Replagal also differed at the COOH-terminus.[24] The α-galactosidase A mRNA has a rather unusual 3' structure with the polyadenylation signal almost directly following the translation termination codon, i.e., little if any 3' untranslated region.[25] However, the RNA contains only a single translation terminus. Consequently, variation in the COOH-terminus derives from posttranslational proteolytic processing. The predicted COOH-terminal would include the sequence LKDLL. Fabrazyme and Replagal contained LKD, LKDLL, and LKDL.[24] The two enzyme preparations contained equal amounts of LKD (approximately 22%) but differ greatly in content of LKDLL (70% vs. 6%) and of LKDL (7.6% vs. 73%), respectively, for Fabrazyme and Replagal. These oligosaccharide and amino acid sequence differences have no apparent effect on basic kinetic ($K_m$, $V_{max}$) or Fabry fibroblast uptake parameters. Fabrazyme had a slightly greater affinity for isolated M-6-P receptors than Replagal.[24]

The enzyme is soluble, and newly synthesized enzyme is trafficked to the lysosome by the mannose-6-phosphate receptor system.[26] Normally, the enzyme is secreted from cells at low levels but can be made to secrete in substantial amounts when overexpressed.[23] The enzyme also requires a cofactor, saposin B, in proper stoichiometry to allow THC to be solubilized from membranes and presented to the enzyme for catalysis.[27,28] The enzyme is present in all tissues as a normal step in the catabolic pathway for glycosphingolipids. The apparent selective and differential/cell-type accumulations of THC in various tissues are not understood. A significant amount of THC is secreted by the liver in association with low density lipoprotein (LDL) and very low density lipoproteins (VLDL) particles, but the other sources of the THC remain to be defined. The differential glycosphingolipid contents of blood-formed elements have been suggested to account for some differences between α-galactosidase A deficiencies in humans and mice,[29] and this suggests a role of these elements in disease development. In addition to THC, blood-group substance B has a terminal α-galactosyl moiety that also is a substrate for α-galactosidase A.[28] As such, blood-group B+ individuals with Fabry disease may have a more rapidly progressive course than patients with other blood types.

The nature of the mutations, the presence of residual activity, the ABO blood-group type, and other genetic factors may have substantial influence on the clinical heterogeneity of Fabry disease patients. Even among classical Fabry patients with no detectable enzyme activity or protein, there is significant variation in the age of onset, rate of progression, and eventual organ manifestations.[8] We have observed substantial differences in disease manifestations between hemizygous males within a single family, indicating that the gene mutation accounts for only a part of the variation of this disorder. The basis of this heterogeneity must be taken into account when considering treatment trials of rare diseases because smaller studies, in general, may have significant selection bias if the patients are related, or if a group of patients have a particular set of manifestations. This heterogeneity is not understood but clearly is a significant problem related to assessment of disease progression and response to therapy. Substantial individual variation in degree of involvement and response to ET is well recognized and poorly understood in ET for Gaucher disease.[1]

## PRECLINICAL PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES IN FABRY MOUSE MODELS

Until recently, the only extensive characterization of α-galactosidase A isoforms in the Fabry mouse model was published by Ioannou et al.[30] None of these glycoforms were structurally characterized in detail and, moreover, were derived from different CHO clones than Fabrazyme. The study compared four different variants of human α-galactosidase A from CHO having greater or lesser sialylation or differing amounts of mannose-6-phosphate residues. The carbohydrate content apparently did not have significant influence on the biodistribution or the pharmacokinetics of α-galactosidase A. In general, one would have anticipated that the more highly sialylated forms would be retained longer in the plasma than lesser sialylated forms; more highly sialylated glycoproteins generally have fewer mannose-6-phosphate residues for uptake by that system. Independent of the sialylation, the $t_{1/2}$ for plasma clearance of the various forms was biphasic with $t_{1/2}$ approximately 2 to 5 minutes for a rapid phase and $t_{1/2} > 2$ hours for the second phase. More highly sialylated variants had a second phase $t_{1/2}$ that had approximately a 1.5- to 2-fold increase compared with that for lesser sialylated variants. Some minor physical/chemical differences were noted among the "sialylated" and "under mannose-6-phosphorylated" forms. In comparison, Fabrazyme and Replagal at equivalent doses had rapid phase disappearance in plasma with $t_{1/2} = 16.2$ and 6.2 minutes, respectively.[24] The slower phases were, respectively, approximately 41 and 24 minutes.[24] In Ioannou et al.[30], more heavily sialylated variants with substantial mannose-6-phosphorylation had a $t_{1/2}$ in the liver and spleen of about 50 to 60 hours. With equivalent doses, the $t_{1/2}$ values for the two commercial forms were greater than 24 hours in these organs with nearly 100% of the 1 hour α-galactosidase A levels being maintained at 24 hours. In the heart and kidney, the $t_{1/2}$ values were approximately 1 to 2 hours but with a prolonged second phase with ($t_{1/2} > 24$ hours). In general, Replagal levels were approximately 50% of Fabrazyme levels in all tissues after injection of equivalent doses. The exception was the liver, where nearly identical levels were achieved and maintained.[24]

For the study by Ioannou et al.[30], only 25 to 30% of the total injected dose of any of the isoforms could be recovered at 1 hour postinjection. Of the total amount recovered, about 95% was in the liver and was distributed between hepatocytes and Kupffer cells. A dose of 10 mg/kg per dose of the more highly sialylated variant with about six M-6-P residues per dimer increased the amount of detectable enzymatic activity found in the heart and lungs of α-galactosidase A deficient mice. Also, there was an increase (approximately 2-fold) in the total amount of kidney and splenic α-galactosidase A above that detected with 1 mg/kg per dose. The biodistribution of all the variants was independent of the sialylation or M-6-P content. The majority of administered enzyme was taken up into the liver but with detectable activity in the spleen and kidneys. When yeast mannans were used to block the macrophage mannose receptors, the $t_{1/2}$ in plasma of administered α-galactosidase A was increased substantially. Because mannans bind only to mannose receptors, these findings implicate both the mannose-6-phosphate and the mannose receptors in enzyme uptake. Because mannose-6-phosphate receptors are known to be present on macrophages and Kupffer cells,[31] the appearance of α-galactosidase A in such cells could have occurred by mannose-6-phosphate receptors alone. Similar detailed studies are not available for the commercial products, except as noted above.

Reaccumulation of the THC in tissues was also studied following administration of enzyme.[30] Following enzyme infusions, plasma levels of THC decreased from elevated to normal levels, and substantial decreases of THC were found in the liver, spleen, kidney, and heart even though detectable enzyme activity above baseline was not found in these latter two organs at the time of sampling. Plasma THC reaccumulation preceded that in tissue, suggesting a substantial component of the substrate delivered or stored in the various tissues of these Fabry mice was derived by de novo synthesis in the liver and delivered to the tissues by way of circulating lipoprotein particles. These data suggest a balance between THC synthesized in the liver for delivery to tissues and endogenous tissue THC synthesis leading to tissue accumulation. The relative proportions of these sources of THC in Fabry disease are unknown. Thus, delivery of the enzyme to the liver may substantially deplete the stored substrate and thereby diminish (by yet to be described mechanisms) the delivery of THC to the peripheral circulation and subsequently to peripheral tissues. This, in addition to the direct targeting of the enzyme to involved tissues (i.e., endothelial cells, kidney, cardiomyocytes, etc.), may potentate the effects of ET in Fabry patients. Whether accumulated THC in various tissues can reenter the plasma once depleted (i.e., kidney-stored lipid returning to the plasma) was not answered by these studies. We are left to assume that the loss of accumulated lipid in the tissues is due primarily to their hydrolysis by α-galactosidase A delivered to those peripheral tissues. Thus, ET may alter the balance between liver-synthesized and tissue endogenously synthesized THC and the amount of retained activity from administered enzyme. These studies also demonstrated that each of these effects on stored lipid and histologic changes in the Fabry mouse were proportional to dose.

## PRECLINICAL HUMAN TRIALS

### Pharmacokinetics/pharmacodynamics

The available data on either Replagal or Fabrazyme for pharmacokinetics/pharmacodynamics (PK/PD) are limited. Direct comparison of the results of the standard PK parameters showed the $T_{max}$ to be near the end of the infusion time. The area under the curves (AUCs) for Replagal were proportional to dose, whereas those for Fabrazyme were not.[4,32] It is difficult to directly compare Replagal and Fabrazyme for AUCs because the doses were as units/kg per dose or mg/kg per dose, respectively. If one assumes that the two pure preparations have the same specific activity (approximately $3 \times 10^6$ nmol/hr/mg en-

zyme), the Replagal AUC trials were conducted with about 0.05 to 0.2 mg/kg. The Fabrazyme trials were with 0.3 to 3 mg/kg. Thus, the dosing in these studies may differ by at least an order of magnitude. Fabrazyme appeared to saturate, although it may not have achieved a steady state. The clearance was probably by saturable and unsaturable mechanisms. Interestingly, the clearance of both drugs was approximately 1 to 3 mL/min/kg, and the disappearance values $t_{1/2}$ were about the same. Similarly, Vss values were approximately 1 to 4 blood volumes for each drug. Direct comparisons with comparable masses of enzyme would be useful.

Direct comparisons for PK of Fabrazyme and Replagal in the $\alpha$-galactosidase A null mice and in normal monkeys were reported at a meeting in Denmark by the Genzyme Group headed by Tim Edmunds. Using equivalent masses of the two products, the plasma AUC was about 2.5 to 2.8 times greater ($\mu$g min/mL) for Fabrazyme than Replagal. Consistent with this was a clearance that was 2 to 2.8 times (mL/minute kg) faster and a 2-fold increase in Vss (mL/kg) for Replagal. Thus, the comparative data for humans, mice, and monkeys are similar but with some species differences.

For human studies with Replagal, plasma THC was analyzed at 1 hour and 7 and 28 days following infusions of a single dose of enzyme. Dosing ranged from 0.3 units/kg to 4.7 units/kg. The highest dose corresponds roughly to a dose of 0.1 mg/kg. Major decreases were not seen at 1 week. However, a significant decrease in tissue THC was demonstrated at 28 days.[2] As an indirect measure of renal tubular THC accumulation, urinary sediment THC was measured and showed a 38% mean decrease ($P < 0.01$). All but one patient showed a decrease (range 67% decrease to 14% increase). Liver THC levels decreased in 9 of 10 patients (range 14–71%). One patient on a low dose (0.6 units/kg) showed an increase in liver THC, the same patient that had a low change in urinary sediment THC. Another patient receiving a higher dose (4.7 units/kg) demonstrated an increase in urine THC with a decrease in liver THC. By immunohistochemistry, the distribution of Replagal was predominately to Kupffer cells and sinusoidal endothelial lining cells. Some enzyme was detected in hepatocytes.

Fabrazyme was tested using three different doses and at different dosing schedules.[4] The dose ranged from 0.3 to 3 mg/kg per dose every 2 weeks or once every 48 hours. For biweekly injections, a dose-dependent change was found in plasma THC with 0.3 mg/kg per biweekly dose, leading to normalization by the fifth injection. With 1 or 3 mg/kg per dose, two of three or three of three patients, respectively, reached normal levels of THC by the second injection. One patient with 1 mg/kg per dose had a slight decrease in plasma THC. Liver THC levels decreased by 24 to 100% at all doses. In seven patients who had biopsies, a mean increase of 9.6% was detected in myocardial THC. Curiously, variable effects were found in myocardial THC (range from 26 decrease to 93% increase of initial). A mechanism to explain this finding would be rather involved and may reflect sampling artifacts in small numbers rather than pathophysiology. Renal biopsies showed a 97 to 58% decrease of THC, with one patient showing a very large increase (200%). This individual also had a 93% increase in myocardial THC and only an 88% decrease in liver THC. Such variation could be explained by nonuniform variation in THC deposition in different cells and tissues whose contribution would reflect their composition in the biopsy and the potential differential effects of enzyme or THC in various cells types (see below). Such effects would affect the statistics on small numbers of patients.

Similar to Replagal, Fabrazyme was distributed to Kupffer cells, sinusoidal endothelial cells, and to hepatocytes. Significant depletion of accumulated THC was found in liver, renal, and myocardial endothelial cells as well as other cellular types. Little to no change was indicated by histology in renal podocytes. In longer-term studies, only a trend to decreased podocyte THC was documented with Fabrazyme.

### Comparison Of Replagal and Fabrazyme study structures

The study structures for Fabrazyme and Replagal treatment of Fabry disease differed in their primary and secondary end points and their overall designs(Table 2).[2,3] Major differences in the two trials included the single-center (Replagal) or multicenter/multinational (Fabrazyme) designs along with single-site (Replagal) or multisite (Fabrazyme) evaluations in the trials. With Fabrazyme, pathologists with expertise in particular tissues (renal, cardiac, or skin) were blinded not only to treatment but also to the results of the other pathologists. For the Replagal trial, two blinded pathologists reached a consensus as to the score for each of the biopsies. The exact scoring system for the Replagal trial was not made explicit. For the Replagal trial, the primary end point was a change in neuropathic pain, whereas in the Fabrazyme trial, it was the percentage of patients reaching a zero score for endothelial THC deposits. Additional measurements were made of GFR (Replagal and Fabrazyme), renal histology (Replagal), and endothelial clearance

**Table 2**
Replagal and Fabrazyme clinical trial structures

| Replagal | Fabrazyme |
|---|---|
| 29 Fabry hemizygotes | 56 Fabry hemizygotes + 2 affected heterozygotes |
| Single center | Multicenter/multinational |
| Randomized placebo controlled | Randomized placebo controlled |
| Primary endpoint, BPI pain assessment | Primary endpoint, renal histologic change (percent of patients achieving 0 score) |
| Secondary endpoint | |
| Renal histology, multiple cell types | McGill Pain inventory |
| Renal function, inulin/creatinine clearance | Renal function, inulin/creatinine clearance |
| Plasma/urine THC | Plasma/urine THC |
| Quality of life | Quality of life |
| | Additional renal histology |

of THC deposits in skin and heart (Fabrazyme) to supplement primary end points. Other secondary end points for Replagal and Fabrazyme were quality of life and THC in urinary sediment, plasma, and tissues. Additional results of more extensive evaluation of renal histology are now available for the Fabrazyme study (see below).

The phase 3 study of Fabrazyme included 58 patients (56 male, 2 female). Randomized treated patients received 1 mg/kg per dose over 4 to 6 hours every 2 weeks. Renal, endomyocardial, and skin biopsies were done at baseline and 5 and 11 months. The latter was included for the crossover from placebo to Fabrazyme. Pain scores were assessed using the McGill pain scale at the entry and at the close of the study.

The phase 3 Replagal trial included 26 hemizygous males selected for neuropathic pain. The primary measure of the study was an assessment of a change in the pain severity. Renal biopsies were done at baseline and 6 months. Enzyme infusions (0.2 mg/kg) were given over 40 minutes every 2 weeks. Effects of enzyme infusions on kidney and heart disease as well as quality of life were assessed.

Consideration of pain measurements is important because pain is a significant problem during daily activities and for quality of life for affected individuals. The chronic neuropathic pain and episodic pain crises in patients with Fabry disease have proven refractory to standard treatments, although some relief is possible with neuroleptic medications and narcotics. The measures of pain in both trials differed in important ways. The Replagal trial included pain assessment at each infusion. This could tend to create memory of the pain scales and, therefore, measure relatively short-term perception of pain. Also, the withdrawal of pain medications would create an anticipation of pain that also could "train" the patient to tolerate additional pain levels. The Replagal study included nine questions with a scale of 1 to 10, with 10 as the most severe possible pain and 1 being pain free. Patients were required to forego medication at weeks 8, 16, and 23 for as long as possible following the measures of their pain. The pain measure for the Fabrazyme study was done at the beginning and end of the study only, making tool memory less likely. These patients remained on their medications, regardless of pain status at study entry. The McGill pain scale is a 45 point scale where higher scores indicate more severe pain; the scores were relatively low (5–8 of 45), indicating mild to moderate levels of pain. Whether these were qualitatively more or less than in the Replagal study cannot be ascertained from the presented data.

#### Assessment of meeting primary end points

For Replagal, the Brief Pain Inventory (BPI) "Pain at its Worst" scores for patients were thought to be statistically different for those receiving enzyme infusions versus those receiving placebo. The mean pain score at baseline and at weekly intervals were significantly different in the treatment versus placebo groups. Indeed, at each time point, the BPI severity or BPI pain related quality of life measurements differed between the group by about 1.9 or 1.6. Thus, at baseline, the placebo group by either criteria had higher pain scores than did the treatment group, and this difference was maintained. This represents a significant skewing of the initial data in the placebo group (i.e., this group had more/worse pain). The reason for this difference is not clear. Because a difference in score of 1 between the groups was stated to be statistically significant, a 1.6 to 1.9 point difference between the groups is highly significant and confounding. If the greater pain (i.e., higher score) is significantly more difficult to change (i.e., the pain response change discrimination is less for greater pain), then the study is inherently biased in favor of the test group in the evaluation of pain outcome. Stated differently, if the more intense pain is less likely to change or to be perceived as a change than a lower pain score, then inherently no change in the placebo group would have been noted unless it were dramatically different when compared with a lower pain threshold group. A divergence of the test and placebo group pain scores would have been expected if the drug had a significant effect on pain perception. However, the changes in pain reports were parallel throughout the study, supporting some confounding effect. In addition, inherent in the discontinuation of pain medication as a response characteristic is the possibility that, by chance, individuals would have discontinued pain medication on their own. Thus, the lack of a "run-in" trial to assess who would have come off of medication spontaneously, or had chosen to discontinue their medication at any given time, would have been important to evaluate this particular aspect of the study. The fact that no patient in the placebo group of 11 taking neuropathic pain medications was able to discontinue these pain medications may have no meaning for comparative purposes in a trial in which the placebo group had substantially more pain than the treatment group. An important aspect of these studies would have been to assess whether a placebo group with comparable initial pain scores would have been able to remain off pain medication for the same periods of time "mean values" as the Replagal treatment group. Equally confounding is the apparent decrease of pain in the placebo group in parallel with the test group over the course of the study. This may relate to the structure of the pain assessments as indicated above. The decrease in pain measurements may indicate a possible benefit but should not be considered a proven benefit of treatment until a difference has been demonstrated between a treatment group and controls with equivalent levels of pain at the time of study entry.

The pain scores in the Fabrazyme study were equivalent, but relatively low, for both the treatment and placebo groups. Each group demonstrated a significant decrease in pain scores, indicating a placebo effect. Also, two assessments are insufficient to demonstrate differences between groups with relatively mild pain at the start of treatment.

On the basis of the published data from both trials, further study with longer follow up involving greater numbers of patients and using similar/identical assessment tools will be needed to establish the effect of ET on pain. An additional confounder is the potential misassessment of Fabry pain by available instruments. Personal observations (GAG and RH) indicate the following: many Fabry patients have indicated that

the vocabulary on the pain scales does not adequately or accurately express or evaluate their various types of pain. Moreover, they indicate the existence of several varieties of pain, some are, and others are not, responsive to either neuroleptics, narcotics, or ET. Additional bias may enter studies if patients are skewed into a group representing a responsive or nonresponsive type of pain. This also would effect the time off medication. Thus, more disease-specific tools may be needed to reflect the actual clinical response of the patient's pain. Such an approach has more general implications.

Also, the value of pain assessments must be evaluated as a clinical tool for therapeutic effect of ET in Fabry disease. Acroparesthesias and other types of pain are observed in female heterozygotes frequently (approximately 70–80%). In comparison, renal (nonpodocyte) histologic abnormalities, clinical decreases in renal function, and frank progression to end-stage renal disease are unusual (3–10%). Thus, the occurrence or intensity of pain does not correlate in a predictable manner with the extent nor degree of renal impairment. Consequently, elimination of pain is a needed and important goal of ET for Fabry disease, but treatment of the lethal renal complications of this disease is essential to this patient population's health improvement.

#### Assessment of renal pathology and function

Figure 5 shows schematically the structures of the kidney and the location of some cell types analyzed in the two studies. Changes in microvascular endothelial cell THC deposits in the kidney were a primary endpoint for the Fabrazyme trial (interstitial capillaries) and supporting data for the Replagal trial (glomerular capillaries). In the Fabrazyme trial, three blinded pathologists each scored interstitial capillary endothelial cell deposits for an average of 233 capillaries in each renal-biopsy specimen. To reach the primary end point of efficacy, more than 50% of the renal interstitial capillaries had to score 0 (normal), and less than 5% could had to have a score of 1 or greater (progressively greater THC deposits). The remainder (approximately 45%) would need to be designated as having trace evidence of endothelial cell THC deposits at the end of the trial. A majority score was determined from the three scores. At baseline, the Fabrazyme and placebo groups had scores of $1.9 \pm 0.8$ and $2.2 \pm 0.7$. These values were not statistically different. By week 20, the Fabrazyme and placebo groups had scores of $0.4 \pm 0.7$ and $2.1 \pm 0.8$, respectively (i.e., there was no difference between baseline and week 20 in the placebo group, but an average change of $-1.6 \pm 1.2$ was present in the Fabrazyme group with Fabrazyme); 20 of 29 reached the endpoint with scores of 0 (69%) compared with 0 of 29 in the placebo group. Of the remaining eight, six showed improved scores and two had not changed. This was highly statistically significant. In a crossover extension trial, additional clearance was seen in the original test group, and an equally robust response (90–100% patients with 0 scores) was observed in those crossed over from placebo. The scoring system developed, and its blinding to observer and test versus placebo addresses the inherent potential for observer and sampling bias. In addition, the large number of analyses directly addresses the sample bias, but an evaluation of the number and distribution (random?) of unanimous scores (13/3) versus the majority (2/3) would have provided added strength to the conclusions. Overall, these data strongly support a direct effect on THC storage in these renal cells of patients receiving $\alpha$-galactosidase A because they were based directly on the response of the test group.

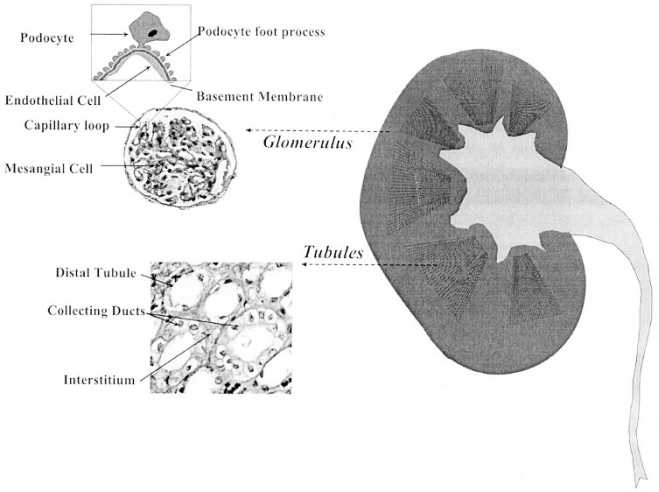


**Fig. 5** Drawing of the kidney and some morphologic/cellular features evaluated in the Replagal and Fabrazyme trials. Shown schematically are the locations in the kidney of the various features. Most are from the cortical areas, but biopsies can sample various areas with or without some features.

For Replagal, a different scoring system was used that was not explicitly delineated and, therefore, not directly comparable with the Fabrazyme trial. The glomerulus was emphasized in the Replagal trial with evaluations of the numbers and types of glomeruli with or without mesangial widening, presence or absence of glomerulosclerosis, or the presence of obsolescent (i.e., globally sclerotic) glomeruli. Tubular interstitial fibrosis, hyalinosis, and tubular atrophy also were scored. In comparing baseline with week 24 of Replagal therapy, significant differences were seen in the glomeruli, with mesangial widening and segmental sclerosis between the treatment versus placebo groups ($0.01 > P < 0.05$). Similarly, the numbers of normal glomeruli were different pre- and posttherapy in the treatment and placebo groups. Inspection of the initially reported data indicated a substantially larger number of normal glomeruli ascertained in the placebo group (59.6) compared with the treatment group (39.9). The difference at 24 weeks was caused by a decrease of 16 in the placebo group versus an increase of 8 in the Replagal group. The mesangial widening was substantially different in the Replagal group (38.2) versus the placebo group (23.9) at the start of the study. The differences between the two groups at 24 weeks were due mainly to the decrease in the mesangial widening in the treatment group to nearly the baseline placebo levels and an increase in placebo group mesangial widening to nearly baseline levels in the Replagal treatment group. Segmental sclerosis was greater in the placebo group versus the treatment group at baseline, increased substantially in the treatment group, and decreased significantly in

the placebo group by week 24. No difference was found in the number of obsolescent glomeruli. Vascular endothelial inclusions showed a decrease ($P < 0.002$) in the treated group.

Concordant with the changes in renal histology in the Fabrazyme trial was a decrease in the amount of THC accumulated in the kidney. The Fabrazyme treatment group showed a 23.3% decrease in THC, whereas the placebo group showed a 42.8% increase in kidney biopsies. Similarly, a decrease in urinary-sediment THC was found in the treatment group (34.1%) compared with a 6.2% decrease in the placebo group. The statistical confidence was not indicated. In the Replagal trial, similar changes were not observed. The mean renal THC did decrease slightly, 4.0 nmol/mg of tissue versus 0.9 in the placebo group, but this was not statistically significant. Urinary sediment THC decreased in the Replagal group (approximately 30%) and increased in the placebo group (15%, $P = 0.05$).

More detailed analyses of the renal biopsies from patients receiving Fabrazyme revealed heterogeneity in the degree of cellular response by THC clearance.[33] By using a similar scoring system to that in the pivotal trial for interstitial endothelial cell scoring, comparisons were made of THC-present endothelial cells of the glomerulus, peritubular capillaries, and arteries/arterioles as well as vascular smooth muscle cells, podocytes, distal collecting tubules (DCT), and mesangial cells. Comparisons for each of these cell types were made in patients receiving Fabrazyme for either 6 or 11 months for whom complete biopsy sets were available. For all groups listed above except vascular smooth muscle cells, DCT, and podocytes, from 69 to 100% of patients achieved 0 scores and 86 to 100% had 1 to 3 point reductions in scores at either 5 or 11 months. In vascular smooth muscle cells, 0 to 10% achieved 0 scores, but 81 to 86% achieved a 1 to 3 point reduction in score. For DCT, 25 to 78% achieved significant reductions. The podocytes responded poorly, with 5 to 24% reductions by 6 to 11 months of Fabrazyme. These additional data indicate that at the appropriate dose of $\alpha$-galactosidase A, complete clearance of stored THC can be achieved in many renal cell types, except podocytes and DCT where there was a trend toward improvement.

Renal function was assessed by inulin or creatinine clearance in each study. The Replagal trial showed a significant difference in the inulin clearance changes in the treatment and placebo groups. A significant decline in the placebo group from a mean of 90.9 to 71.5 mL/min/1.73 $m^2$ (mean values) was reported, whereas there was no change in the Replagal treatment group (mean of 77.2 vs. 70.0). The ranges, however, were different. At least one patient receiving placebo showed a 70 mL/min/1.73 $m^2$ decrease in inulin clearance during the treatment period (i.e., at least one patient progressed to renal failure in the placebo group). No statistics are provided for this group without this individual(s), or if this individual(s) would account for the changes in the placebo group. In the Fabrazyme study, the summary statement on GFR indicated no change in either group. No follow-up data were provided in this trial, although no difference was indicated in GFR over the 6-month open-label period. For either study, it would have been useful to have presented the primary data on the GFRs for each individual patient so that evaluations could be more carefully considered. The Replagal trial is confounded by the presence of at least one individual that entered renal failure in the placebo group, the greater mean GFR in the placebo group (13 mL/min/1.73 $m^2$), and at least one patient in the treatment group who had a 28 mL/min/1.73 $m^2$ decrease in GFR. Similar data are not available for the Fabrazyme trial. The dichotomy of results in the placebo groups of the two studies requires comment. In the Replagal study, the placebo-group GFR data imply a progression of renal disease that was not apparent in the comparable group in the Fabrazyme trial. This raises the issue of the potential heterogeneity within these aggregate GFR data in the Replagal trial vis-à-vis the individual(s) who experienced the dramatic drop(s) in GFR. If the impact of this patient(s) was not great, then, together with the higher pain score, this may indicate a different subset of Fabry patients in the placebo group in this study. More individualized data would help resolve these perplexing issues.

#### Other organ assessments

Tabulation of additional endothelial changes in the heart and skin were provided in the Fabrazyme trial, and these reached statistically significant differences using the same type of scoring system as was used for kidney interstitial endothelial cells. The heart and skin endothelial-cell deposits are cleared by Fabrazyme treatment, and this was highly statistically significant. In the Replagal trial, the prolonged electrocardiographic wave (QRS)-complex duration decreased significantly ($P$ approximately 0.05), and an abstract indicates a separate trial where cardiac mass decreased by 4% in one patient.[34] This has not been evaluated thoroughly. No assessments of cardiac mass were presented in the Fabrazyme trial.

#### Plasma THC clearance

Because of the accessibility, both studies evaluated plasma THC clearance. Both found statistically significant differences between the placebo and treatment groups ($P$ approximately 0.005). Neither placebo group showed a change in plasma THC levels. In the Replagal trial, a 54% decrease was found by week 24 in the treatment group. The plasma THC levels dropped to undetectable levels (25 patients, $<$1.2 ng/mL) or decreased somewhat (88–16%) by week 20 in Fabrazyme patients. The effect of enzyme dosage on plasma THC levels in Replagal group (0.2 mL/kg) versus Fabrazyme (1 mg/kg/dose) is consistent with the preclinical dose-response trial.[4] Importantly, the majority of patients in either trial developed immunoglobulin (Ig)G antibodies to the administered enzyme. The persistent decrease in plasma THC in the majority of patients suggests that these antibodies did not have significant inhibitory effects on the enzyme in vivo.

The CNS pathology in Fabry disease is significant and probably underappreciated.[35–37] However, neither study incorporated formal CNS evaluations. Replagal has been reported to normalize cerebral blood flow in Fabry hemizygotes.[38,39] The authors initially hypothesized that there would be decreased perfusion secondary to microvascular occlusion (because of

endothelial storage of THC); however, positron emission tomography (PET) scans revealed increased cerebral blood flow. Additional studies implicated involvement of oxidative stress through the nitric oxide pathway in causing the over perfusion. The cerebral hyperperfusion and dermal nitrotyrosine staining normalized after 6 months of ET. This normalization of cerebral blood flow as assessed by PET may be evidence of decreased risk for stroke with enzyme replacement but is at this point a preliminary result of unknown significance.

Different quality of life measures were used in each study, and both studies reported changes only in subscales of quality of life. The Fabrazyme study reported improved quality of life in physical role and emotional role for treated patients and improvements in physical role and body pain in the controls using the 36-item Medical Outcomes Study Short-form General Health Survey (SF-36). The Replagal study reported improved scores in pain related quality of life as measured using the BPI. Unfortunately, the differences between the treatment and placebo groups make this finding difficult to interpret. The Replagal study also reported an average weight gain of 1.5 kg in the treated group compared with a 1.4 kg weight loss in the controls. The Fabrazyme study did not report weight changes. Neither study addressed the gastrointestinal symptoms (frequent abdominal pain, diarrhea, and probable malabsorption), although this represents a significant morbidity associated with Fabry disease. Only anecdotal evidence of improvement in the peripheral neuropathy was presented, with possible improvement in sensation and sweating claimed for Replagal.[40] Similar reports are available from patients receiving Fabrazyme but have not been included in the published reports (G.A. Grabowski and R.J. Hopkin, personal observation).

### Safety

ET for Fabry disease is associated with a relatively high risk for allergic reactions. However, both Fabrazyme and Replagal were generally well tolerated. Unfortunately, it is not possible to directly compare much of the safety data because of differences in antibody detection methods. The sensitivity of the various assays for immunoglobulin (Ig)G antibodies were not explicitly described, and without such information, the frequency of antibody positivity cannot be compared.

The Replagal study reported mild infusion reactions in 57% (8/14) of patients who received the protein. The most frequent symptoms were rigors that were treated with antihistamines, corticosteroids, or by slowing the infusion rates. The rate of infusion was decreased midway through the study to decrease the number if infusion related reactions. No serious reactions were reported. Patients were tested for antibody formation at baseline and at 9, 17, and 24 weeks. No patients developed IgE antibodies. Eighty-six percent had some level of detectable IgG [(including both measurable antibody titers (3/14) and positive immunoprecipitation assays (9/14)]. The IgG reactivity tended to decrease with time, suggesting tolerization.

The Fabrazyme study reported mild to moderate infusion reactions in 59% of treated patients. The reactions were treated with slowing infusion rates and medications including antihistamines. All patients were tested for antibody formation before each infusion. Eighty-eight percent (51/58) of patients developed detectable IgG. No patient experienced a significant complication of treatment. Antibody titers tended to decrease with time, suggesting tolerization. One patient became skin-test and IgE positive after eight infusions and was discontinued from therapy. Treatment has been reinstituted under controlled conditions and has been well tolerated. Thus, ET for Fabry disease appears to be safe and well tolerated with either Fabrazyme or Replagal. Nevertheless, patients will need to be carefully monitored, especially when initiating therapy, because a small number of patients may eventually develop serious allergic reactions. This may relate to the absence of any residual enzyme activity or protein in classical Fabry disease patients. In comparison, about 15% of Gaucher disease patients develop allergic symptoms, and about half appear to be antibody mediated.[41–43] About 12% of Gaucher patients develop IgG antibodies to the infused enzyme, and all are directed to polypeptide epitopes.

Review of the published studies of Fabrazyme and Replagal reveal weaknesses in each study. Unfortunately, the differences in study design make direct comparison of efficacy impossible. Both drugs have demonstrated clearance of THC from plasma and histologic evidence of improvement on a tissue level. This makes it very likely that ET for Fabry disease will be an effective treatment. Neither drug has demonstrated a clear improvement for pain or renal function. This is probably because of the short study duration, the small numbers of treated patients, and the potentially irreversible nature of the organ damage. It seems clear that ET will not reverse existing renal failure or completely eliminate pain, although it is likely to delay or slow progression. The effect of ET on cardiac function, risk for stroke, and the peripheral neuropathy of Fabry disease requires additional study. Another area in critical need of study is the natural history of Fabry disease in heterozygous women.

## CONCLUSIONS

ET for Gaucher disease clearly opened the successful era of specific treatment of lysosomal-storage diseases by protein replacement. However, because of the rarity of the lysosomal diseases, the lack of thorough documentation of their natural history, and the appreciation of their heterogeneity, a decade has been needed to establish the therapeutic response characteristics. Clearly, the saga with Fabry disease is just beginning, but the lessons of the progress with Gaucher disease and the need for cooperative development of standards of assessment are clear and promising for Fabry disease patients. On the basis of the reversal of the pathologic accumulation of THC in Fabry patient tissues, particularly renal tissue, the prospects for stabilization and potential reversal appear likely for some or much pathology and for progression of this disease. Clearly, earlier intervention and determination of enzyme dosage for best therapeutic effects will require long-term studies with detailed follow-up of appropriate clinical, functional, and histo-

logic measures of disease involvement. The prospects for effective therapy and improvement in the health of Fabry disease patients have been increased greatly by the extension of ET with this disease.

The safety and efficacy of Fabrazyme and Replagal were reviewed by an FDA Advisory Panel on January 13th and 14th, 2003. The briefing documents are available at www.fda.gov/ohrms/dockets/ac/acwhatsnew.htm (Endocrinologic and Metabolic Drugs Advisory Committee).

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