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ORIGINAL ARTICLE BRIEF REPORT

An Atypical Variant of Fabry's Disease with Manifestations Confined to the Myocardium

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FABRY'S DISEASE is an X-linked recessive disorder resulting from deficient activity of the lysosomal hydrolase α-galactosidase A.[1–3] The enzymatic defect leads to the progressive accumulation of neutral glycosphingolipids with terminal α-galactosyl moieties (particularly globotriaosylceramide) in the lysosomes of vascular endothelial and smooth-muscle cells throughout the body. In classically affected males, who have no detectable α-galactosidase A activity, the onset of disease manifestations occurs in childhood or adolescence and is characterized by severe acroparesthesias, angiokeratoma, corneal opacities, and hypohidrosis. The cardiac manifestations result from the accumulation of globotriaosylceramide in the myocytes, leading to myocardial failure; in coronary endothelial cells, resulting in myocardial infarction; and in valvular fibroblasts, leading predominantly to mitral insufficiency.[4–10] Hypertrophic obstructive cardiomyopathy and the development of a pattern of dilated cardiomyopathy have been described.[11,12] Affected hemizygotes may have angina, a short PR interval,[13] ST-segment changes,[14] or all three features. The course of the disease is progressive, and with advancing age complications of myocardial, renal, or cerebral vascular disease may occur in male patients. Before the introduction of renal transplantation and hemodialysis, the average age of affected hemizygous males at death (usually caused by renal failure) was 41 years.[1] With successful treatment of the renal insufficiency, subsequent disease manifestations result from slowly progressive myocardial infiltration. In contrast, men with atypical variants of the disorder, who have residual α-galactosidase A activity, are asymptomatic or have mild symptoms.[1,15–21] Heterozygous female carriers of the disease-causing gene usually have no symptoms or minimal disease involvement and have a normal life span.

We report a variant of Fabry's disease that is apparently limited to the myocardium. The patient had unexplained angina pectoris, normal coronary arteries and hemodynamic findings, and typical lysosomal inclusions in an endomyocardial-biopsy specimen, but none of the other pathological or clinical findings of the disease. The recent isolation and sequencing of the full-length complementary DNA and entire genomic sequence encoding human α-galactosidase A[22,23] have facilitated the characterization of the molecular lesions causing the classic and variant forms.[24–27.] Applying these techniques, we identified a missense mutation in exon 6 of the α-galactosidase A gene, which encoded an enzyme with residual activity in this atypical patient with isolated myocardial disease.

Case Report

The proband, a 54-year-old man, was admitted to the hospital because of crescendo angina that had occurred during the previous two weeks. The pain was relieved by nitroglycerin. There was a history of a "common cold" four weeks before admission. For 13 years, mild allergic bronchial asthma without any respiratory complications had been treated with theophylline (500 mg daily), fenoterol (1.2 mg daily), and cromolyn sodium (40 mg daily by nebulizer). Risk factors for coronary heart disease were hyperlipidemia, diabetes mellitus (treated by diet only), and a questionable history of systemic hypertension. The patient's father had diabetes, his mother had died of uterine carcinoma at the age of 58, and two maternal uncles, a maternal aunt, and a maternal female cousin died of cardiac disease before 50 years of age. His full brother and half brother (through his mother) were in good health. On physical examination, the patient was obese (82 kg; 1.65 m), with hypertension mm Hg) and tachycardia (105 beats per minute). Cardiac auscultation gave normal

results. Slit-lamp examination did not reveal any corneal opacities, and angiokeratoma was not observed on careful examination. There was no history of acroparesthesias or hypohidrosis.

Laboratory studies demonstrated that the hemoglobin level was 9.9 mmol per liter g per deciliter), the hematocrit 46.2, the leukocyte count 6000 per cubic millimeter, the erythrocyte sedimentation rate 18 mm per hour, the serum creatinine concentration 110 μmol per liter (1.2 mg per deciliter), and the creatinine clearance 1.6 to 1.8 ml per second (96 to 108 ml per minute) per 1.73 m^2 of body-surface area. Urinalysis revealed proteinuria (0.32 to 0.52 g per day). The levels of electrolytes in serum and urine were normal. Cholesterol concentrations ranged from 8.7 to 9.8 mmol per liter (337 to 378 mg per deciliter) (normal, ⩽5.7 mmol [220 mg]), and triglyceride concentrations from 4.1 to 9.2 mmol per liter (363 to 817 mg per deciliter) (normal, ⩽2.3 mmol [200 mg]). The concentration of low-density lipoprotein cholesterol was 6.0 mmol per liter (232 mg per deciliter) (normal, 2.3 to 4.9 mmol [90 to 190 mg]), and that of high-density lipoprotein cholesterol was 1.3 mmol per liter (49 mg per deciliter) (normal, 0.9 to mmol [35 to 75 mg]). The serum glucose concentration ranged from 6.4 to 15.5 mmol per liter (115 to 279 mg per deciliter) during the day. The results of serum protein electrophoresis were normal. The total serum creatine kinase activity was μkat per liter (135 U per liter), of which 0.1 μkat per liter (6.2 U per liter) represented the activity of the MB isozyme. Chest x-ray films and an intravenous pyelogram were normal. Electrocardiography revealed inverted T waves in leads V_5 and V_6 and left anterior hemiblock. M-mode and two-dimensional echocardiography showed that the left and right ventricles and atria were of normal size and had normal systolic and diastolic function. Mild left ventricular hypertrophy was present (posterior-wall thickness during diastole, 12 mm). Myocardial perfusion imaging with thallium-201 showed a persistent defect in the posterior left ventricular wall. Cardiac catheterization was performed, since coronary heart disease or perimyocarditis was suspected as a possible diagnosis. There were no stenoses of the extramural coronary arteries, and left ventricular function at rest was normal (end-diastolic pressure, 11 mm Hg; ejection fraction, 86 percent; cardiac output, 6.7 liters per minute). Mild pulmonary hypertension was detected (mean pressure, 26 mm Hg). An endomyocardial biopsy was performed. Two bicycle exercise tests had to be stopped at workloads of 75 and 125 W because of exhaustion; no angina or ST-segment depression occurred. After Fabry's disease was diagnosed, biopsies of the skin, liver, skeletal muscle, and rectum were performed with informed consent.

The patient's medical condition has remained stable for the past four years, with occasional mild angina not specifically related to physical activity. Further episodes of crescendo angina have not occurred. A second skin biopsy, performed during a recent operation for bilateral carpal tunnel syndrome, revealed no ultrastructural evidence of Fabry's disease. No angiokeratoma or corneal opacities have developed, and renal function has remained normal (creatinine [illegible] per liter), with nearly normal protein excretion (0.32 g per day). Electrocardiographic examination gave normal results except for mild left ventricular hypertrophy. An exercise test (100 W) revealed no angina pectoris or ST-segment depression. A chest film was normal. Renal biopsy was refused by the patient.

Methods

CELL LINES

Skin specimens and peripheral-blood samples were obtained from the proband and members of his family with their informed consent. Cultured fibroblast and lymphoblast cell lines were established and maintained according to standard techniques.[24]

MORPHOLOGIC STUDIES

Tissue obtained for light-microscopical investigation was fixed in formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin. For electron microscopy, specimens were fixed in glutaraldehyde (6.25 percent), postfixed in 2 percent buffered osmic acid solution for two hours, and embedded in Epon. Semithin sections were stained in methylene azure II, and ultrathin sections were subjected to contrast staining with uranyl acetate and lead citrate.

BIOCHEMICAL AND MOLECULAR STUDIES

The levels of α-galactosidase A activity in plasma and cell sources were determined with the fluorogenic substrate methylumbelliferyl-α-D-galactopyranoside (Genzyme, Cambridge, Mass.).[3] One unit of enzymatic activity is the amount required to hydrolyze 1 nmol of substrate per second under the conditions of the assay. Normal and residual α-galactosidase A activity in 10^8 cultured lymphoblasts was partially purified by affinity chromatography[28] for kinetic, stability, and immunologic studies. Thermostability was determined by incubation of the enzyme with human serum

albumin (1 mg per milliliter) at 40°C and pH 4.6 in 0.2 M sodium acetate, or at 40°C and pH in 0.1 M HEPES buffer (Calbiochem—Behring, La Jolla, Calif.). Monospecific polyclonal rabbit antihuman α-galactosidase A antibodies (50 ng) were used to estimate the amount of α-galactosidase A enzyme protein by immunoprecipitation of a standard amount (0.26 unit) of partially purified αgalactosidase A activity from lymphoblasts of the proband and normal subject.[29] Total protein was measured by the fluorescamine assay.[28] Neutral glycosphingolipids were measured as described.[30,31]

Southern and Northern hybridization analyses were performed as previously described.[24,32,33] First-strand complementary DNA was reverse transcribed from approximately 10 μg of total RNA, isolated from the proband's lymphoblasts,[32,33] with the BRL cDNA Synthesis Kit (Bethesda Research Laboratories, Gaithersburg, Md.). The entire α-galactosidase A complementary DNA was amplified by the polymerase chain reaction[34] in two overlapping fragments using two sets of primers: the set 1 sense primer was 5′-ACTACTGAATTCGCTGCTCGGTCACCGTG-3′, and the antisense primer was 5′-ATGTACGTCGACAGATGTCCAGTCCAAGATAC-3′; the set sense primer was 5′-ACTACTGAGCTCCAATTATACAGAAATCCGACAGT-3′, and the antisense primer was 5′-GTCATGGTCGACCTTTTAATGACATCTGC-3′. Double-stranded template was isolated from six independent pGEM-4Z subclones of each product of the polymerase chain reaction, and each was sequenced in both orientations by the dideoxy chain-termination method[35] with the use of universal and α-galactosidase A—specific primers.

To confirm the point mutation identified in the reverse transcribed and amplified α-galactosidase A transcript, a region of genomic DNA from the proband and from a normal subject that contained exon 6 was amplified with the polymerase chain reaction as described above, by means of the sense and antisense primers 5′ -GGTACCTAATACGACTCACTATAGGGAGAGCTCTGGGTCATCTAGGTAACT-3′ and 5′-ACTACTGTCGACCTGATAGTAACATCAAG-3′, respectively. After phenol—chloroform extraction and ethanol precipitation, the amplified DNA was sequenced directly according to a modification of the method of Wong et al.[36] with an end-labeled oligonucleotide 5′-ATATGTTAGTGATTGGC-3′) that contained the first 12 bases of exon 6 as a primer. The secondary structure of the normal and mutant enzymes was predicted by the method of Chou and Fasman[37] with the use of software from the University of Wisconsin Genetics Computer Group.[38]

Results

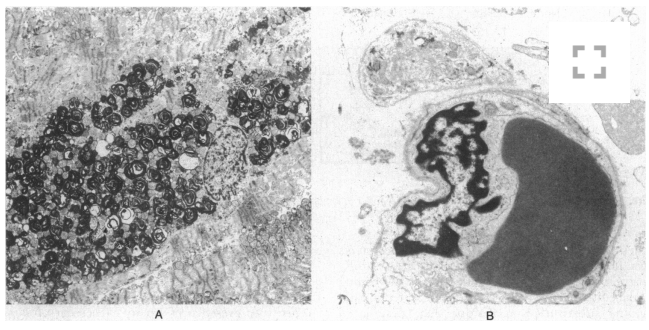
MORPHOLOGIC STUDIES

Light-microscopical examination of a diagnostic endomyocardial-biopsy specimen revealed that approximately half the myocytes contained a centrally stored foamy material that stained metachromatically. Ultrastructurally, typical myelin-figure—like concentric lamellar inclusions in lysosomes were observed (Fig. 1A), a finding consistent with the diagnosis of Fabry's disease. Most remarkably, the endothelial cells of the myocardial capillaries were not involved (Fig. B). A second endomyocardial-biopsy specimen, obtained five months later, showed identical findings, as assessed independently by two pathologists. No evidence of storage material was observed on histologic or ultrastructural examination of specimens of skeletal muscle, liver, rectum, and skin, including small blood vessels and nerves.

BIOCHEMICAL AND MOLECULAR STUDIES

The pathological diagnosis of Fabry's disease was confirmed by the demonstration of decreased α-galactosidase A activity in various cells and sources from the proband (Table 1). Residual enzymatic activity was present, ranging from about 1 to 25 percent of the respective mean values in normal sources. It was notable that the residual activity was highest in plasma and cultured lymphoblasts (12 percent and 25 percent of mean normal values, respectively). The α-galactosidase A activity in cultured lymphoblasts from the proband and from normal subjects was partially purified (about 25-fold) by affinity chromatography for thermostability and kinetic studies. As compared with the purified normal enzyme, the residual enzyme in the proband was markedly less stable after preincubation at 40°C at the optimal pH for the enzyme, 4.6 half-time, 37 vs. 12 minutes), or at a pH of 7.4 (halftime, 52 vs. 10 minutes). In addition, the purified residual activity had a slightly higher apparent K_m value (Michaelis constant) than the purified normal enzyme (3.8 vs. 2.8 mM) toward the fluorogenic substrate. Immunoprecipitation studies indicated

FIGURE 1



Electron Photomicrographs of the Endomyocardial-Biopsy Specimen from the Proband with Fabry's Disease. Panel A shows a heart muscle cell filled with single membrane-bound vacuoles (i.e., lysosomes) containing electron-dense concentric lamellar inclusions (×2300). Panel B shows a myocardial capillary, without lysosomal inclusions in the endothelial cell ×10,000).

that the decreased activity in the proband resulted from a decreased amount of enzyme protein. The plasma globotriaosylceramide level in the proband was only slightly higher than the levels in his unaffected full and half brothers, which were repeatedly found to be greater than the upper limit of normal (Table 1). In addition, the globotriaosylceramide concentration in urinary sediment from the proband was only slightly above the normal range, suggesting that only low levels of the substrate were present in the kidney.[39]

Southern and Northern hybridization analyses revealed no gross α-galactosidase A—gene rearrangements and normal size and abundancy of the transcript (data not shown). Therefore, to identify a putative point mutation or small insertion or deletion, total cellular RNA was isolated from the proband's lymphoblasts, the α-galactosidase A messenger RNA was reverse transcribed with a primer corresponding to the 3′ end of the transcript, and then the complementary DNA was specifically amplified in two overlapping fragments by the polymerase chain reaction. As shown in Figure 2, nucleotide sequencing of independent pGEM-4Z subclones containing the products of the polymerase chain reaction revealed an A-to-G transition at nucleotide 886 in exon 6 of the coding sequence in all clones, which predicted a methionineto-valine substitution at residue 296 (designated M296V). This missense mutation was confirmed in genomic DNA by polymerase-chain-reaction amplification and direct sequencing of an exon region including this nucleotide, as well as by dot—blot analysis using allele-specific oligonucleotides (data not shown). The A-to-G transition in nucleotide 886 was present in the proband, but the normal sequence was found in both his brothers. Computer-assisted analysis[37,38] of residues 276 to 316 of the α-galactosidase A subunit predicted that the methionine-to-valine substitution (M296V) would replace a region of random coil with a β-pleated—sheet motif in the enzyme's secondary structure.

TABLE 1

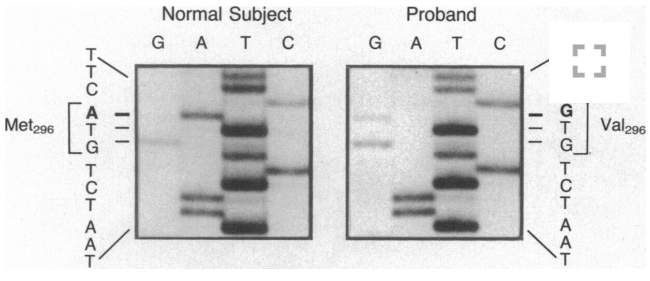
Table 1. Levels of α-Galactosidase A Activity and Concentrations of Globotriaosylceramide in Members of the Proband's Family and Other Groups.*

Source	Proband	Full Brother	Half Brother	Normal Subjects	Patients with Classic Fabry's Disease	[illegible] al
α-Galactosidase A						
Plasma (U/liter)	0.40	2.42	1.64	3.22 (1.7–7.9)	<0.06	12.5
Fibroblasts (U/g)†	2.51	ND	ND	27.4 (20.9–39.7)	<0.6	9.2
Lymphoblasts (U/g)†	4.36	33.3	43.3	17.4 (3.86–46.1)	<0.14	25.1
Lymphocytes (U/g)†	0.36	11.6	7.58	9.06 (5.97–13.9)	<0.06	4.0
Granulocytes (U/g)†	0.26	14.3	12.3	18.9 (12.2–24.8)	<0.06	1.4
Globotriaosylceramide						
Plasma (nmol/liter)	4220	3960	3610	2690 (1620–3740)	10,100 (6720–15,400)	157
Urinary sediment (nmol/day)	64.0	ND	ND	24.0 (7–30)	1570 (240–4910)	267

*ND denotes not determined. †Per gram of protein.

Levels of α-Galactosidase A Activity and Concentrations of Globotriaosylceramide in Members of the Proband's Family and Other Groups.

FIGURE 2



Partial DNA Sequence of α-Galactosidase A Exon 6, Indicating the A-to-G Transition at Nucleotide 886 (M296V).

Discussion

Atypical variants of Fabry's disease with sufficient residual α-galactosidase A activity to prevent or markedly delay the major manifestations of the disease have been previously described.[15–21] The first variants reported were observed in patients who did not have angiokeratoma, the dermatologic hallmark of the disease.[15–17] Subsequently, variants with minimal or essentially no clinical manifestations were observed. For example, two unrelated men and 42 years old) described independently[1,15,19] did not have angiokeratoma, corneal opacities, acroparesthesias, hypohidrosis, or cardiac disease, but had proteinuria that led to renal biopsies and the ultrastructural findings of Fabry's disease. A 26-year-old Japanese man had only severe acroparesthesias and no other clinical features of the disease.[20] In addition, a 51-year-old Arab man who had no disease manifestations was serendipitously identified.[21] Each of these atypical hemizygotes had residual α-galactosidase A activity in all cells examined. Presumably, the atypical phenotypes result from different mutations in the α-galactosidase A gene that encode kinetically altered or less stable enzymes (e.g., as previously reported[24,27.]). The altered enzymes apparently have sufficient residual α-galactosidase A activity to protect such persons from the major morbid manifestations of the disease.

In this communication we report an α-galactosidase A missense mutation (M296V) in an atypical patient with Fabry's disease whose manifestations, which were limited to the myocardium, occurred long after most classically affected patients would have died.[1] In contrast to previously described atypical patients, this diabetic patient had only mild proteinuria, with protein levels near the normal range and no evidence of impaired renal function. Most notable was the absence of lysosomal glycosphingolipid accumulation in endothelial cells of endomyocardium, liver, skeletal muscle, rectum, and skin. This finding may reflect the presence of up to 25 percent of normal mean α-galactosidase A activity in

various sources as well as the low level of globotriaosylceramide in plasma (Table 1). The plasma globotriaosylceramide concentration may have been slightly elevated in the proband and his half brother because of their hyperlipidemia,[40] since both had increased levels of total cholesterol and slightly increased levels of low-density lipoprotein cholesterol.

Myocardial involvement in this and other variants may be due to "mild" α-galactosidase A mutations by the following pathophysiologic mechanism. In patients with the classic form — men with no α-galactosidase A activity — the myocardium is an early site of glycosphingolipid deposition, as evidenced by the accumulation of this lipid in the heart and kidneys of affected fetuses.[41] With age, the progressive myocardial deposition is manifested first by hypertrophy and then by dilatation, both being aggravated by additional valvular abnormalities. However, most patients with the classic form die of complications of the renal and vascular involvement before the myocardial manifestations become debilitating. In contrast, patients with atypical forms may have sufficient residual α-galactosidase A activity to protect the kidneys and vascular endothelium, but the level of enzyme activity in the heart may be inadequate to prevent the progressive deposition of glycosphingolipid in myocardial cells. In later adulthood, myocardial disease develops in these patients, leading to a clinical phenotype of Fabry's disease confined to the myocardium. In support of this concept, three other patients with Fabry's disease limited to the heart have been identified at autopsy.[42,43] Two Japanese patients who died at 58 and 71 years of age had marked deposition of globotriaosylceramide (100 to 340 times normal) in the heart; only one had a slight (twice normal) accumulation in the kidneys. A Czechoslovakian patient had stable angina for 15 years before he died at The autopsy findings in these patients were unremarkable except for the involvement of the myocardium. Presumably, the very late development of myocardial symptoms in heterozygous females who have low levels of α-galactosidase A activity (due to random X-inactivation) also results from this pathologic process.

The findings in the atypical variant described here, together with the recent findings in several other cases,[42,43] indicate that Fabry's disease can occur in which manifestations are limited to the heart. Clearly, patients with cardiac symptoms (angina, exercise intolerance, and electrocardiographic changes) but normal coronary arteries, heart size, and hemodynamic findings present a frequent and usually unresolved problem to every cardiologist. Thus, Fabry's disease should be considered as a possible cause of otherwise unexplained cardiac symptoms. Such cases may be identified by ultrastructural examination of endomyocardial-biopsy specimens or, less invasively, by determining the plasma α-galactosidase A activity in male patients with unexplained cardiac symptoms.

NOTES

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REFERENCES

1. Desnick RJ, Bishop DF. Fabry disease: α-galactosidase A deficiency; Schindler disease: α-N-acetylgalactosaminidase deficiency. In: Scriver C, Beaudet A, Sly W, Valle D, eds. The metabolic basis of inherited disease. 6th ed. New York: McGraw-Hill, 1989:1751-96.
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2. BradyRO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease: ceramidetrihexosidase deficiency. *N Engl J Med* 1967; 276:1163-7.
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3. Desnick, RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W. Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes: α-galactosidase activities in plasma, serum, urine, and leukocytes. *J Lab Clin Med* 1973;81:157-71.
+ Show Citations PubMed Google Scholar

4. Ferrans VJ, Hibbs RG, Burda CD. The heart in Fabry's disease: a histochemical and electron microscopic study. *Am J Cardiol* 1969; 24:95-110.
↵ Go to Citation Crossref PubMed ISI Google Scholar

5. Desnick RJ, Blieden LC, Sharp HL, Hofschire PJ, Moller JH. Cardiac valvular anomalies in Fabry's disease: clinical, morphologic, and biochemical studies. *Circulation* 1976; 54:818-25.

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