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Fabry disease: Detection of undiagnosed hemodialysis patients and identification of a "renal variant" phenotype[1]

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Fabry disease: Detection of undiagnosed hemodialysis patients and identification of a "renal variant" phenotype.

Background. Fabry disease is an X-linked recessive lysosomal storage disease resulting from deficient α-galactosidase A (α-Gal A) activity. Renal failure is a major debilitating complication in classically affected males. To determine if this disorder is underdiagnosed in patients with end-stage renal disease (ESRD), the frequency of unrecognized males with Fabry disease on chronic hemodialysis was determined.

Methods. Plasma α-Gal A activity was measured in 514 consecutive males with ESRD on hemodialysis. Patients with low α-Gal A activity were evaluated clinically and their α-Gal A mutations were determined.

Results. Six (1.2%) of 514 hemodialysis patients had low plasma α-Gal A activities and a previously identified (E66Q, A97V, M296I) or novel (G373D) missense mutation. At ages 30 to 68 years, five patients lacked the classic manifestations of angiokeratoma, acroparesthesias, hypohidrosis, and ocular opacities, while the sixth lacked angiokeratoma and ocular changes. Five had left ventricular hypertrophy (LVH).

Conclusion. The clinical spectrum of Fabry disease includes a "renal variant" phenotype in patients without classic symptoms who develop ESRD. Affected males undergoing hemodialysis or renal transplantation can be readily diagnosed by plasma α-Gal A assays. These patients and their family members may benefit from enzyme replacement therapy for the later, life-threatening cardiovascular and cerebrovascular complications of Fabry disease.

Fabry disease is an X-linked recessive lysosomal storage disorder resulting from the deficient activity of the lysosomal hydrolase, α-galactosidase A (α-Gal A) [1–3]. The enzymatic defect leads to the progressive accumulation of glycosphingolipids, predominantly globotriaosylceramide (GL-3), throughout the body, and particularly in blood vessels, kidney, and heart [3–6]. Affected males who have little, if any, α-Gal A activity exhibit the "classical" Fabry phenotype with onset of acroparesthesias, angiokeratoma, hypohidrosis, and corneal and lenticular opacities in childhood or adolescence. With advancing age, the progressive lysosomal GL-3 accumulation, particularly in the vascular endothelium, leads to renal failure, vascular disease of the heart and brain, and premature demise. In contrast, female carriers for the classic phenotype have a range of clinical involvement due to random X-inactivation [7], ranging from most being asymptomatic or having mild manifestations to rare carriers having as severe disease as affected males [3].

Recently, a variant form of Fabry disease was identified with manifestations primarily limited to the heart [8–11]. These "cardiac variants" lack the classical disease symptoms, and present in the sixth or seventh decade of life with left ventricular hypertrophy (LVH) and/or cardiomyopathy. They may have proteinuria, but their renal function is typically normal for age. Of note, "cardiac variants" have residual α-Gal A activity due to missense mutations and lack the systemic vascular endothelial glycosphingolipid deposition characteristic of classically affected patients [3]. Screening of 230 consecutive Japanese male patients with LVH and 153 British male patients with hypertrophic cardiomyopathy by plasma α-Gal A assays revealed that 3% and 3.9%, respectively, were previously unrecognized "cardiac variants" [11, 12].

Early diagnosis of Fabry disease is important because it permits family studies to identify other affected relatives for genetic counseling and therapeutic intervention. This is especially true now that clinical studies have shown the safety and effectiveness of enzyme replacement therapy for Fabry disease [13–16], as well as the potential for enzyme enhancement therapy [17, 18]. En-

[1] **See Editorial by Grünfeld, p. 1136.**

Key words: Fabry disease, lysosomal storage disease, α-galactosidase A deficiency, end-stage renal disease, hemodialysis, mutation detection, genotype/phenotype.

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zyme replacement therapy is currently available in many countries and is pending approval in others. However, because Fabry disease is not common or well known and its early classical manifestations tend to be nonspecific, the disorder often is unrecognized, misdiagnosed, or diagnosed late in life [3, 19].

To determine if Fabry disease is underdiagnosed among male patients with end-stage renal disease (ESRD), efforts were undertaken to screen patients in chronic hemodialysis clinics in the Japanese prefectures of Kagoshima and Miyazaki. In this communication, we report that six (1.2%) of 514 consecutive male hemodialysis patients had deficient α-Gal A activity and Fabry disease. Notably, five of the six patients did not have the classical disease manifestations, including acroparesthesias, angiokeratomas, hypohidrosis, or corneal and lenticular lesions. These patients extend the clinical spectrum of Fabry disease and identify a "renal variant" phenotype.

METHODS

Patients

A total of 514 consecutive, unselected Japanese male patients with ESRD treated with chronic hemodialysis at clinics in the Kagoshima and Miyazaki Prefectures were screened with informed consent for Fabry disease by measuring their plasma α-Gal A activities. The patients ranged in age from 20 to 90 years (mean ± 1 SD, 54 ± 17 years).

Clinical evaluation

The six patients with abnormally low plasma and lymphocyte α-Gal A activities were evaluated for the clinical manifestations of classical Fabry disease, including angiokeratoma, acroparesthesias, hypohidrosis, corneal and lenticular opacities, and cardiomegaly by clinical history, physical examination, slit-lamp microscopy, and echocardiography. The criterion for LVH was a ventricular septum and/or posterior wall thickness of at least 13 mm [20]. In addition, the family members of patients 5 and 6 were examined clinically, and urinary protein, serum creatinine, and plasma α-Gal A activity were determined. Of note, only 16 (3.1%) of the 514 patients had renal biopsies before chronic hemodialysis, including patient 6, who had a biopsy 15 years prior to this study for evaluation of his proteinuria. This biopsy was routinely fixed, stained with periodic-acid Schiff (PAS), and examined by light microscopy. For this study, the original PAS-stained slides were reexamined, and the original biopsy specimen was osmium-fixed, embedded in epoxy-resin, cut, and stained with toluidine blue O for light microscopy. In addition, ultrathin sections, cut and doubly stained with uranyl acetate and lead citrate, were examined by transmission electron microscopy.

Measurement of α-Gal A activity and GL-3 levels

Plasma α-Gal A activity was measured before hemodialysis in all 514 patients with renal failure and was assessed in 89 healthy Japanese male subjects ranging from 14 to 80 years of age (mean ± 1 SD, 52 ± 19 years) who served as controls. Plasma or lymphocyte α-Gal A activity was determined with the fluorogenic substrate, 4-methylumbelliferyl-α-*D*-galactopyranoside (Sigma Chemical Co., St. Louis, MO, USA) as previously described [3], except that the reaction mixture included 500 μmol/L of α-*N*-acetyl-D-galactosamine (Nacalai Tesque, Kyoto, Japan) to inhibit the α-*N*-acetylgalactosaminidase activity [21]. Plasma α-Gal A activities of ≤2.5 U/mL were defined as abnormally low. Lymphocyte α-Gal A activity was determined in the six patients who had low plasma α-Gal A activity, as well as in 43 healthy Japanese male subjects ranging in age from 24 to 46 years. A unit (U) of enzymatic activity is that amount of enzyme required to hydrolyze 1 nmol of substrate per hour at 37°C. Plasma GL-3 levels in ng/mL were determined as previously described [22].

Mutation analysis

Genomic DNA was isolated from whole blood with the DNA Extractor WB Kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions. Mutation analysis was performed by polymerase chain reaction (PCR) amplifying each of the seven α-Gal A exons and their flanking intronic sequences from genomic DNA using one biotinylated primer in each primer set. A single biotinylated strand was isolated using streptavidin-coated magnetic beads and then sequenced by the solid-phase direct method as previously described [23]. Each mutation was confirmed by repeat PCR amplification and sequencing of the opposite strand. A mutation was considered disease-causing whenever there was cosegregation of the lesion and disease phenotype or low α-Gal A activity in each family.

RESULTS

Abnormally low plasma α-Gal A activities (<2.5 U/mL) were found in six (1.2%) of the 514 male patients with renal failure who were not previously diagnosed as having Fabry disease. Enzymatic activities in the six patients ranged from 0.6 to 2.4 U/mL, ~7% to 28.6% of the mean activity in 89 healthy males (mean ± 1 SD, 8.4 ± 2.4 U/mL; range, 4.8 to 17.9 U/mL). The plasma α-Gal A activities in the remaining 508 patients ranged from 4.1 to 29.1 U/mL (mean ± 1 SD, 9.2 ± 3.7 U/mL) (Fig. 1). Lymphocyte α-Gal A activities in the six patients ranged from 0.9 to 12.9 U/mg protein, ~2% to 28% of the mean activity in 43 healthy male controls (mean ± 1 SD, 46.4 ± 6.5 U/mg protein; range, 33.4 to 60.9 U/mg protein). The plasma GL-3 levels were elevated in all six patients,

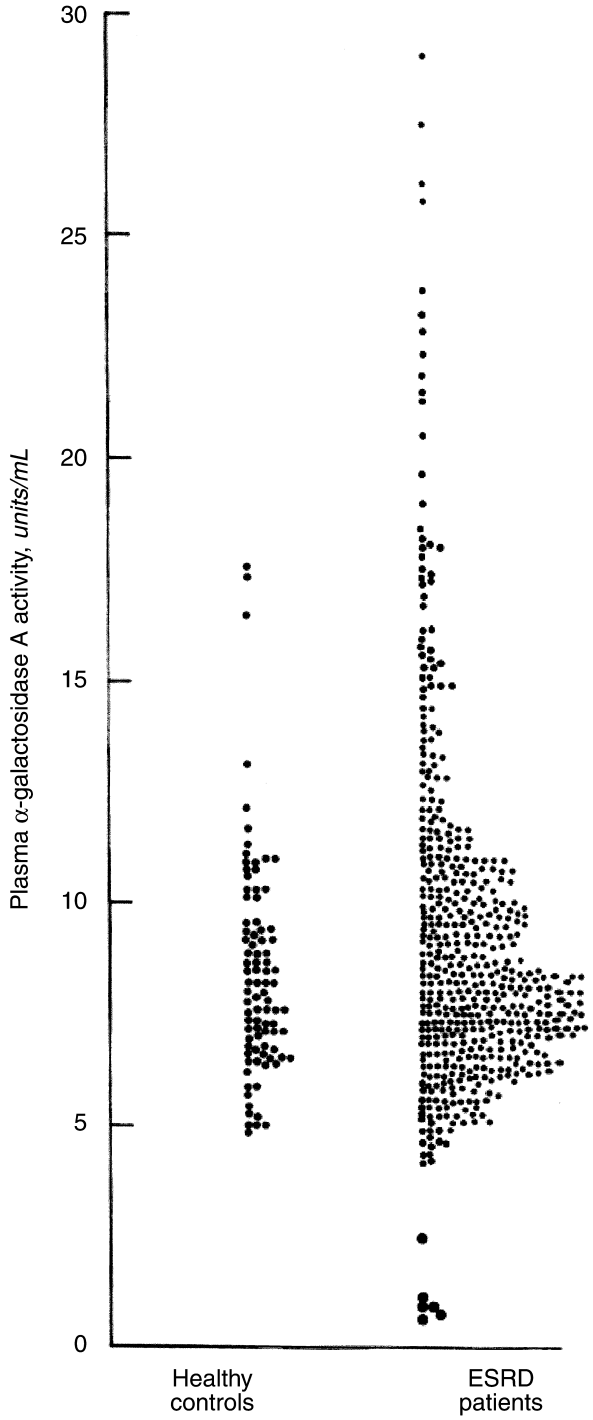

Fig. 1. Plasma α-galactosidase A (α-Gal A) activity in 89 normal healthy males and 514 males with end-stage renal disease (ESRD) on hemodialysis. See accompanying text for details.

ranging from 3.6 to 17.7 ng/mL, compared to the normal mean value of 2.1 ng/mL (range, 1.2 to 2.8 ng/mL SD ± 0.7) [22].

The six male patients with low α-Gal A activities ranged in age from 30 to 68 years, were not related by history, and initiated hemodialysis at 25 to 56 years of age. Each had been diagnosed with chronic glomerulonephritis. Among the 366 screened patients classified as having chronic glomerulonephritis, 1.6% actually had Fabry disease. Of the other screened patients with a specific diagnosis, no Fabry patients were identified. Notably, angiokeratoma and corneal and lenticular opacities were not observed in any of the six patients. Acroparesthesias and hypohidrosis were reported only by patient 6. All but patient 4 had LVH with left ventricular thickness ranging from 14 to 20 mm (Table 1).

Reevaluation of patient 6's original PAS-stained renal biopsy specimen revealed subtle foamy inclusions in the podocytes, consistent with glycosphingolipid deposits that had been "leeched-out" during fixation. Subsequent fixation of the original biopsy in osmium to preserve the glycosphingolipid deposits and staining with toluidine blue O revealed dark-blue stained granular deposits of the glycosphingolipid in the epithelial cells and glycosphingolipid inclusions in the capillary endothelial cells of the glomerulus (Fig. 2A). On electron microscopy, stacked or concentric lamellar figures, or solid osmophilic-dense material was observed in the epithelial and endothelial cells, consistent with the glycosphingolipid deposits characteristic of Fabry disease. Of note, patient 6 had a family history of renal disease. His mother died from renal failure of unknown cause at 40 years of age and his older brother died from renal failure at 42 years of age. Another brother began hemodialysis for renal insufficiency at 34 years of age; his plasma α-Gal A activity was markedly deficient, consistent with the diagnosis of Fabry disease. Like his brother (patient 6), he has acroparesthesias and hypohidrosis, but did not have angiokeratoma or corneal clouding. Patient 6's 34-year-old sister experienced acroparesthesias during adolescence, but did not have angiokeratoma, hypohidrosis, or corneal opacities. She had mild albuminuria, normal serum creatinine (0.4 mg/dL), and normal renal function. Her plasma α-Gal A activity was 6.1 U/mL, consistent with heterozygosity for Fabry disease. Patient 5 had no family history of Fabry disease. His father and mother died at 92 years and at 90 years, respectively. His 34-year-old daughter had albuminuria, a normal serum creatinine (1.1 mg/dL), and decreased plasma α-Gal A activity (2.4 U/mL). She did not have angiokeratoma, acroparesthesias, hypohidrosis, or corneal/lenticular opacities.

Genomic amplification and solid-phase direct sequencing of single-stranded genomic DNA from each of the six patients identified four different missense mutations. Patients 1, 2, and 3 had G to A transitions of nucleotide 888 in exon 6, which predicted the substitution of isoleucine for methionine at residue 296 (M296I). A three-generation pedigree from each of these patients failed to reveal evidence of relatedness. Patient 4 had a G to C transition at nucleotide 196 in exon 2, predicting

Table 1. Clinical, biochemical, and molecular characteristics of hemodialysis patients with the "renal variant" of Fabry disease

Characteristic	Patients					
	1	2	3	4	5	6
Age *years*	30	57	62	68	59	37
Age started hemodialysis *years*	25	47	56	51	56	35
Original diagnosis	CGN	CGN	CGN	CGN	CGN	CGN
Renal biopsy	−	−	−	−	−	+ (glomerulosclerosis)
Left ventricular wall thickness *mm*						
Interventricular septum	15	20	17	11	15	17
Posterior wall	12	20	12	11	14	17
Left ventricular fractional shortening	42	28	30	28	40	41
Angiokeratoma	−	−	−	−	−	−
Acroparesthesias	−	−	−	−	−	+
Hypohidrosis	−	−	−	−	−	+
Corneal/lenticular opacities	−	−	−	−	−	−
α-Gal A activity						
Plasma *U/mL*	1.1	1.1	0.9	2.4	0.7	0.6
Lymphocytes *U/mg protein*	2.7	1.5	3.0	12.9	1.6	0.9
α-Gal A mutation						
Base substitution	G→A	G→A	G→A	G→C	C→T	G→A
Mutation	M291I	M291I	M291I	E66Q	A97V	G373D
Plasma GL-3 *ng/mL*	3.7	6.5	3.8	5.6	6.5	17.7

Abbreviations are: α-Gal A, α-galactosidase A; CGN, chronic glomerulonephritis; GL-3, globotriaosylceramide (normal mean 2.4 ng/mL; range, 1.3–2.8 ng/mL). α-Gal A values in normal males ranged from 4.8 to 17.6 U/mL (mean, 8.4 ± 2.4; $N = 89$) in plasma, and from 33.4 to 60.9 U/mg protein (46.4 ± 6.5; $N = 43$) in lymphocytes.

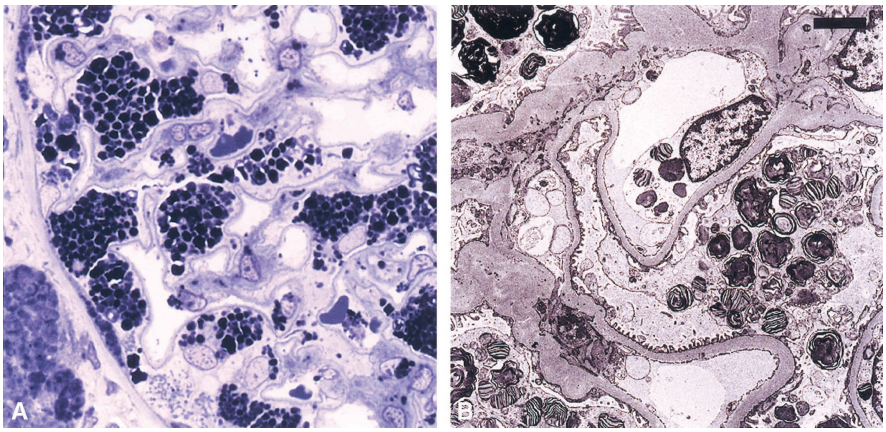

Fig. 2. Photomicrograph of a renal biopsy from patient 6. (*A*) Fixation of the original specimen in osmium and staining with toluidine blue O revealed the glycosphingolipid deposits in the epithelial cells and in the capillary endothelial cells as observed by light microscopy (×780). (*B*) Electron photomicrograph of a uranyl acetate and lead citrate stained ultrathin section showed stacked or concentric lamellar inclusions, and/or solid opaque material in the epithelial and endothelial cells (bar indicates 3 nm).

the substitution of glutamine for glutamic acid at residue 66 (E66Q). The lesion in patient 5 was a C to T transition at nucleotide 290 in exon 2, which predicted the substitution of valine for alanine at residue 97 (A97V). Analysis of genomic DNA from his obligate heterozygous daughter revealed the A97V mutation, as expected. Patient 6 had a G to A transition of nucleotide 1118 in exon 7, predicting the substitution of aspartic acid for glycine at residue 373, a newly identified mutation (G373D). His affected brother and heterozygous sister also inherited the G373D mutation.

DISCUSSION

Almost every lysosomal storage disease has severe early-onset and milder later-onset subtypes with distinct phenotypes resulting from different mutations in the same gene (e.g., types A and B Niemann-Pick disease, infantile and adult Pompe disease, types 1 and 2 Tay-Sachs disease, and mucopolysaccharidosis types IH and IS) [24]. Null mutations cause the most severe phenotypes, while missense, in-frame small deletions, and splicing lesions that encode mutant enzyme proteins with varying levels of residual activity are responsible for the milder subtypes. However, it has become increasingly clear that the phenotypes in each disease are not stereospecific, but rather, that different mutations (or combinations of mutations for autosomal-recessive traits) result in a spectrum of clinical severity for each disease. Such is the case for Fabry disease, with severe classical and distinctively milder cardiac variant phenotypes [3]. Among the over 300 different α-Gal A mutations identified to

date [Human Gene Mutation Database; *http://uwcm.ac.uk/uwcm/mg/hgmd0.html*], patients with the classical phenotype have nonsense, severe missense, frame-shift, and splicing mutations that result in no enzyme protein or mutant enzymes with very low activity (<1% of normal mean). In contrast, all cardiac variants described to date had missense mutations that encoded mutant enzyme proteins or intronic lesions that markedly reduced transcript levels, but resulted in sufficient residual α-Gal A activity (~1% to 10% of normal) to modify the phenotype.

Here, we expand the phenotypic spectrum of Fabry disease to include patients who develop ESRD at ages similar to those of classically affected patients, but lack the other characteristic manifestations of the classic phenotype that usually signal its diagnosis, including angiokeratoma, acroparesthesias, hypohidrosis, and/or corneal and lenticular opacities. Thus, these "renal variants" have a phenotype that is "intermediate," between that of classically affected and cardiac variant patients. Consistent with this concept, the six "renal variants" reported here had four different missense mutations that expressed residual α-Gal A activity, three of which (E66Q, A97V, and M296I) were reported previously, in cardiac variants who did not have renal insufficiency [11, 25, 26]. The fact that the same mutation can occur in different Fabry subtypes is not novel; for example, classically affected and cardiac variants have been described with the R112H and G328R lesions [27–29]. The R112H mutation occurred in a 48-year-old male with the classic phenotype, including impaired renal function [29], and in a 56-year-old male with the cardiac variant phenotype and mild proteinuria [28]. The G328R mutation was identified in a 54-year-old male cardiac variant [29] and in a 34-year-old male with the classic disease manifestations, whose classically affected maternal uncle had developed renal insufficiency [27]. Thus, certain mutations can result in different phenotypes, suggesting that modifying genes and/or environmental factors are involved in determining disease severity. Modifying genes might include those involved in the synthesis of various α-galactosyl-containing glycolipids, including the blood group glycolipids B, B1, and P_1, as the accumulation of these additional α-Gal A substrates may alter disease severity [3, 30].

The diagnosis of Fabry disease is often missed or delayed, particularly in the absence of a previously identified family member [3]. Males with the classic phenotype typically present in childhood with the characteristic angiokeratoma and acroparesthesias. However, if these signs and symptoms are subtle or absent [31], the disorder may not be recognized until adulthood when proteinuria, renal insufficiency, and/or cardiomyopathy are detected and the diagnosis is belatedly made. In contrast, "cardiac variants" have no history of acroparesthesias, do not have angiokeratoma or renal insufficiency, and may be 50 years of age or older before onset of their cardiac manifestations. Thus, these mildly affected patients are easily missed, unless the cardiologist considers this rare variant in the differential diagnosis of LVH, dilated cardiomyopathy, hypertrophic obstructive cardiomyopathy, or idiopathic cardiomegaly [11, 12].

In this report, we further explore the phenotypic spectrum of Fabry disease. By determining the plasma α-Gal A activity in a series of 514 male patients undergoing hemodialysis in Japan, six males (or 1.2%) were found to have a novel presentation of the disease. All six patients had been diagnosed as having "chronic glomerulonephritis," a diagnosis assigned to patients presenting with uremia and a history of proteinuria for 1 year or longer. Of note, none of the six patients had angiokeratoma or the corneal and lenticular dystrophy, which may have led to their earlier diagnosis. Only patient 6 had the classic manifestations of acroparesthesias and hypohidrosis. Of note, his affected younger brother also had acroparesthesias and hypohidrosis, but lacked angiokeratoma and corneal clouding, indicating that the renal variant phenotype bred true in this family. Only patient 6 had a renal biopsy. However, the biopsy was routinely fixed and stained with PAS and examined histologically, and not by electron microscopy, which provides a more definitive diagnosis [3, 32]. In retrospect, the histologic diagnosis could have been made, particularly if the biopsy had been optimally fixed to preserve the glycosphingolipid deposits for staining with toluidine blue O (Fig. 2A), or better with methylene blue-azure II [3].

In this series of 514 hemodialysis patients, only 16 (3%) had renal biopsies, similar to other surveys in Japan where about 5% to 6% of hemodialysis patients with ESRD have renal biopsies [33]. However, it is a general policy in Japan not to biopsy patients who present with ESRD, as was the case for five of the six patients found to have Fabry disease.

Although the incidence of Fabry disease has not been determined in Japan, it is clear from this study that Fabry patients who develop renal insufficiency are missed. Of note, a recent survey of 440 male dialysis patients in the Tokyo area revealed two (0.5%) who had unrecognized Fabry disease, one of which clearly had the classic phenotype [34]. In 2000, there were approximately 120,000 male patients in Japan with ESRD on hemodialysis [33]. If even 0.5% of these patients have undiagnosed Fabry disease, there may be as many as 600 additional patients with Fabry disease in Japan alone. Analogously, in the United States in December 2000, the most recent data available, there were over 125,000 males undergoing hemodialysis. Of these, 26% were diagnosed with hypertension, 12% with glomerulonephritis, and 20% were diagnosed with other unknown causes, but most (>95%) had not had kidney biopsies [35, 36]. Therefore, it is recommended that all patients undergoing renal dialysis

or transplantation without a specific disease diagnosis (i.e., diabetes, polycystic kidney disease), particularly those not having a renal biopsy, be screened for Fabry disease by a plasma α-Gal A assay, which is reliable, relatively simple, inexpensive and available from commercial and academic laboratories (e.g., *http://www.mssm.edu/genetics/fabry*, *http://www.kufm.kagoshima-u.ac.jp/~intmed/*). This approach was recently implemented in Italy, and screening of 1765 male renal dialysis patents identified four (0.23%) who had Fabry disease, with both the classic and renal variant phenotypes [37]. Thus, the frequency of unrecognized Fabry disease among male dialysis patients may vary by racial, ethnic, demographic group, and nephrologic screening from 0.2% to 1%. Since less than 10.0% of female carriers for the classic phenotype develop renal failure [3, 38–40], screening for cryptic carriers among female dialysis patients is likely to be less effective.

CONCLUSION

Fabry disease was identified in 1.2% of male Japanese patients with ESRD who had been previously diagnosed as having chronic glomerulonephritis. Most (83%) of these patients did not have the classic manifestations that could have facilitated their diagnosis. These findings suggest that Fabry disease may be underdiagnosed among renal dialysis and/or transplant patients. In addition, these studies indicate that the early presenting symptoms of Fabry disease such as angiokeratoma and acroparesthesias, may not occur in "renal variants," thereby making their clinical diagnosis difficult. However, these variants can be easily detected by their low to absent plasma α-Gal A activities, and genetic counseling can be offered to them and their extended families. Moreover, recognition of these "renal variants" is important, since they presumably will develop the late, life-threatening cardiac and cerebrovascular manifestations of Fabry disease that may be treated by enzyme replacement therapy [13–16].

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