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Anderson-Fabry Disease: A Multiorgan Disease

Antonino Tuttolomondo*, Irene Simonetta, Salvatore Miceli, Antonio Pinto and Giuseppe Licata

Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo, Italy

Abstract: Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by a deficiency of the enzyme α-galactosidase A. FD causes glycolipids, such as globotriaosylceramide (Gb3), to accumulate in the vascular endothelium of several organs (Fig. **2**), including the skin, kidneys, nervous system, and heart, thereby triggering inflammation and fibrosis. These processes generally result in organ dysfunction, which is usually the first clinical evidence of FD. Patients with classic FD have various symptoms, eg, acroparesthesias, hypohidrosis, angiokeratomas, corneal opacities, cerebrovascular lesions, cardiac disorders, andrenal dysfunction.However, evolving knowledge about the natural course of disease suggests that it is more appropriate to describe FD as a disease with a wide spectrum of heterogeneously progressive clinical phenotypes. Indeed, most female heterozygotes develop symptoms due to yet undetermined mechanisms and a high percentage of females develops vital organ involvement including the kidneys, heart and/or brain about a decade later than males. Renal failure is a serious complication of this disease. Fabry nephropathy lesions are present and progress in childhood while the disease commonly remains silent by routine clinical measures. Early and timely diagnosis of Fabry nephropathy is crucial since late initiation of enzyme replacement therapy may not halt progressive renal dysfunction. This may be challenging due to difficulties in diagnosis of Fabry disease in children and absence of a sensitive non-invasive biomarker of early Fabry nephropathy. Accurate measurement of glomerular filtration rate and regular assessment for proteinuria and microalbuminuria are useful, though not sensitive enough to detect early lesions in the kidney. The principal clinical manifestations in Fabry disease consist of artery associated complications (such as cerebral disease and nephropathy), but the pathophysiology of this specific vasculopathy is unclear. Several studies indicate that the specific vascular lesions that are present in Fabry disease occur as a result of vascular dysfunction with major components being endothelial dysfunction, alterations in cerebral perfusion and a pro-thrombotic phenotype. Fabry cardiac involvement has several clinical manifestations (Table **10**): concentric left ventricular hypertrophy without left ventricular dilation and severe loss of left ventricular systolic function, mitral and aortic valvulopathy, disorders of the atrioventricular conduction or repolarization, and compromised diastolic function. The neurological manifestations of Fabry disease include both peripheral nervous system and CNS involvement, with globotriaosylceramide accumulation found in Schwann cells and dorsal root ganglia together with deposits in CNS neurones. The main involvement of the CNS is attributable to cerebrovasculopathy, with an increased incidence of stroke. The abnormal neuronal accumulation of glycosphingolipid appears to have little clinical effect on the natural history of Fabry disease, with the possible exception of some reported mild cognitive abnormalities. The pathogenesis of Fabry vasculopathy remains poorly understood, but probably relates, in part, to abnormal functional control of the vessels, secondary to endothelial dysfunction as a consequence of α-galactosidase A deficiency. The diagnosis of Fabry disease is made in hemizygous males after the detection of the presence of angiokeratomas (Fig. **19 A**, **B**), irregularities in sweating, edema, scant body hair, painful sensations, and of cardiovascular, gastrointestinal, renal, ophthalmologic, phlebologic, and respiratory involvement. A deficiency of alpha-gal A in serum, leukocytes, tears, tissue specimens, or cultured skin fibroblasts further supports the diagnosis in male patients. Since heterozygous women show angiokeratomas in only about 30% of cases and may have alpha-gal A levels within normal range, genetic analysis is recommended. The resultant storage of undegraded glycolipids leads to the progressive development of potentially life-threatening manifestations affecting multiple organ systems in the body. The Mainz Severity Score Index (MSSI) (Table **12**), a scoring system for patients with Fabry disease has been proven to be representative in patients with 'classic' Fabry disease and may be useful for monitoring clinical improvement in patients receiving enzyme replacement therapy. The MSSI of patients with AFD was significantly higher than that of patients with other severe debilitating diseases.

Keywords: ??????????????????????????????????????.

INTRODUCTION

Lysosomal storage diseases (LSDs) comprise a group of at least 50 distinct genetic diseases, each one resulting from a deficiency of a particular lysosomal protein/activity (Fig. **1**) or, in a few cases, from non-lysosomal activities that are involved in lysosomal biogenesis or protein maturation. Fabry disease is the second most common of the LSDs, after Gaucher disease [1].

Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by a deficiency of the enzyme α-galactosidase A [2]. FD causes glycolipids, such as globotriaosylceramide (Gb3), to accumulate in the vascular endothelium of several organs (Fig. **2**), including the skin, kidneys, nervous system, and heart, thereby triggering inflammation and fibrosis [3]. These processes generally result in organ dysfunction, which is usually the first clinical evidence of FD. Patients with classic FD have various symptoms, eg, acroparesthesias, hypohidrosis, angiokeratomas, corneal opacities, cerebrovascular lesions, cardiac disorders, andrenal dysfunction. The symptoms are progressively debilitating, reduce the ability of patients to attend work or school, and severely affect quality of life (Tables **1**, **2**, **3**).

Although the primary pathologic process - i.e., accumulation of Gb3 - is initiated at early stages in life, the course of the disease, in particular progression to vital organ involvement such as end-stage renal disease and heart failure, can differ widely between patients, even in individuals with the same underlying genotype. A current disease model describes the accumulation of Gb3 in different organ systems as the primary insult, followed by secondary cellular dysfunction, ischemia and fibrosis eventually leading to severe end-organ damage and failure. The process of Gb3 accumulation, starting in the unborn child, can be subclinical for many years until secondary disease processes lead to organ dysfunction and specific pathologies can be detected. It seems to be obvious that the onset of clinically evident dysfunction occurs at a comparable late stage of the disease. Taking this into account, the occurrence of proteinuria and reduction of glomerular filtration rate (GFR) in a Fabry patient

*Address correspondence to this author at the Dipartimento Biomedico di Medicina Interna e Specialistica, Università degli Studi di Palermo (Italy); Tel:/Fax: ??????????????????????????????????????;
E-mail: bruno.tuttolomondo@unipa.it

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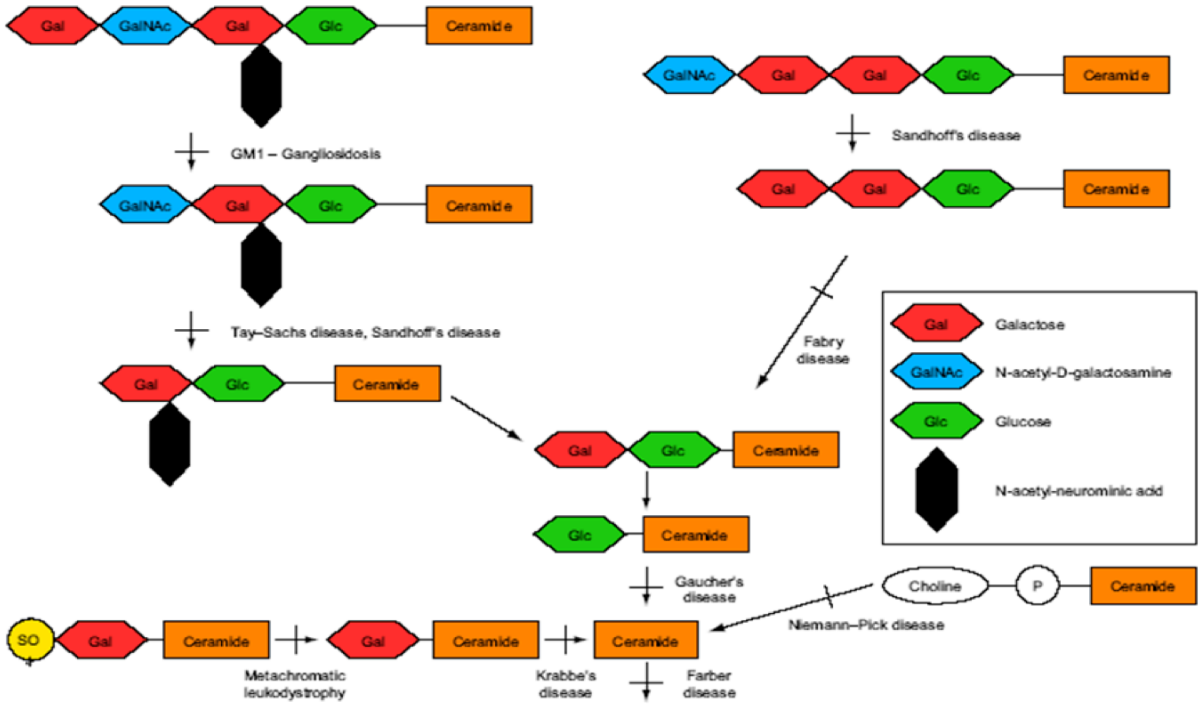


Fig. (1). Lysosomal storage diseases can result from a deficiency of a single lysosomal enzyme.

Table 1. [From Robert J Hopkin, John Bissler and Gregory A Grabowski. Comparative evaluation of α-galactosidase A infusions for treatment of Fabry disease. Genetics in Medicine (2003)]

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

Table 2. [From: Christine M Eng, Dominique P Germain *et al.* Fabry disease: Guidelines for the evaluation and management of multi-organ system involvement. Genetics in Medicine (2006)]

Manifestations of Fabry disease that have an impact on Health-Related Quality of Life

Fabry disease manifestations
General pain
Vertigo
Nausea and/or vomiting
Diarrhea and/or frequent bowel movements
Lack of sweating
Heat/cold intolerance
TIAs
Stroke
Left ventricular hypertrophy
Irregular heartbeat/rhythm disturbance
Heart valve abnormalities
Heart attack
Heart failure
Proteinuria
Abnormal kidney function
Kidney failure

after a stable plateau for 2-3 decades may represent already considerable renal damage leading to ultimate organ failure in a comparably short period of time.

Clinical manifestations in affected hemizygous males are primarily due to progressive disease/involvement of small vessels, including angiokeratoma, autonomic dysfunction, and lifelong debilitating pain. Renal failure and vasculopathy of the heart and brain lead to early demise in adulthood. Demonstration of alpha-galactosidase A deficiency in leukocytes or plasma is the definitive method for the diagnosis of affected hemizygous males. Most female carriers are clinically symptomatic, they may present isolated acroparesthesias, cardiac symptoms, or the characteristic benign corneal dystrophy. Due to random X-chromosomal inactivation, enzymatic detection of carriers is often inconclusive. A reliable molecular test for detection of heterozygosity is therefore highly desirable for accurate genetic counselling. The GLA gene has been mapped to chromosome Xq22, and cloned. Several studies have

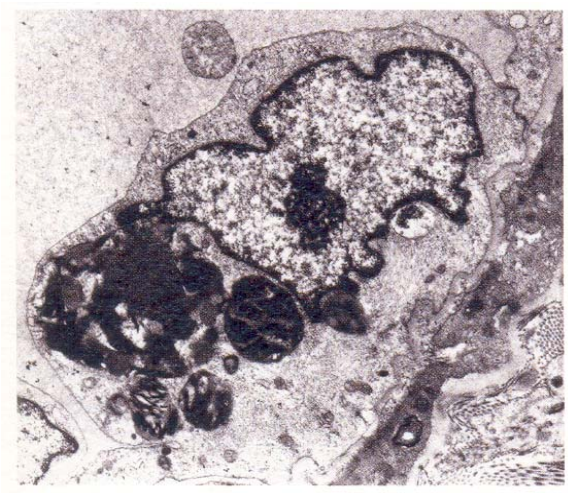


Fig. (2). Endothelial cells (electron microscopy): typical inclusions of AFD(in black), in the lysosomes; they appear thick, lamellar. Courtesy Pr. Massi University of Florense, Italy.

Table 3. [From: Natalie Jansen Street, Michael S Yi, Laurie A Bailey,R J Hopkin. Comparison of health-related quality of life between heterozygous women with Fabry disease, a healthy control population, and patients with other chronic disease. Genetics in Medicine (2006)]

Pathophysiologic findings in Fabry disease

Organ system	Cell types potentially affected by GL-3 deposits	Spectrum of pathophysiologic findings
Kidney	Podocytes, mesangium, glomerular endothelium, epithelium of Bowman's capsule, loops of Henle and distal tubule, arterial and arteriolar smooth muscle and endothelium, interstitial cells[11,92]	Glomerular sclerosis, tubular atrophy, interstitial fibrosis[13,92]
Cardiac	Cardiomyocytes, conduction system cells, vascular endothelial and smooth muscle cells, valvular fibrocytes[93]	LV-hypertrophy; heart failure; stenosis or diffuse narrowing of epicardial vessels; atherosclerotic plaques; coronary vasospasm; thrombotic and thromboembolic complications[9,10]
Neurologic	Neurovascular endothelial cells, vasa vasorum, neurons within central and peripheral nervous system, including dorsal root and autonomic ganglia[94,95]	Ischemic injury (prothrombotic and occlusive abnormalities) and metabolic failure resulting in: Functional disruption of neuronal cells, loss of small myelinated and unmyelinated fibers; ischemic small vessel multifocal leukoencephalopathy; large vessel ectasia[51,78,94]
Dermatologic	Vascular endothelial cells, smooth muscle cells, fibroblasts, perineurium, eccrine sweat glands, including epithelium[64,96,97]	Weakening of capillary wall and vascular ectasia within (epi-)dermis; narrowing of small blood vessels around the eccrine sweat glands[64,65,98]
Ophthalmologic	Epithelial cells in the cornea, lens,[72] vascular endothelial cells[75]	Streaks in corneal epithelium; vasculopathy of the conjunctival and retinal vessels; central retinal artery occlusion; reduced lacrimal secretion[75,78]
Pulmonary	Airway epithelial cells, vascular endothelial cells, smooth muscle cells[62,63]	Airway narrowing, capillary blockage[62]
Gastrointestinal	Vascular endothelial and perithelial cells in the small intestine, colon and rectum; smooth muscle cells; autonomic nerve ganglia in the intestinal wall; small unmyelinated neurons and perineurial cells[55,99]	Narrowing of mesenteric small blood vessels[55]
ENT	Vascular endothelial cells, smooth muscle cells, ganglion cells[48]	Narrowing or total occlusion of cochlear vessels[48]; ischemic auditory neuropathy[48]

shown the molecular heterogeneity of the disease. Currently, no standard treatment exists for Fabry disease. Symptomatic treatment is provided as appropriate. In addition, renal transplantation or dialysis is available for patients experiencing end-stage renal failure.

Over the past 7 years, the Fabry Outcome Survey (FOS) has collected data on the natural history of Fabry disease, and the long-term efficacy and safety of enzyme-replacement therapy. An article by Mehta *et al.* [4] provides an update on the first analysis of FOS data. Baseline data on clinical manifestations and causes of death in a cohort of 1453 patients (699 male, 754 female) from 19 countries worldwide were analysed. Causes of death of affected relatives were analysed separately (Table **4**).

The most frequently reported signs and symptoms of Fabry disease were neurological. Cardiac, ocular, gastrointestinal, dermatological, auditory and renal manifestations were also common. The principal causes of death among 181 affected relatives of patients in FOS (most of whom had died before 2001) were renal failure in males (42%) and cerebrovascular disease in females (25%). In contrast, of the 42 patients enrolled in FOS whose deaths were reported between 2001 and 2007, cardiac disease was the main cause of death in both male (34%) and female (57%) patients. These data suggest that the importance of renal disease as a cause of death in patients with Fabry disease is decreasing while the importance of

Table 4. [From: Atul Mehta and Urs Widmer. Causes of death reported in male (n = 66) and female (n = 38) relatives of patients in FOS - the Fabry Outcome Survey - who are presumed to have had Fabry disease. Natural history of Fabry disease Chapter 19]

Reported Cause of Death	Males	Females
Cardiac	11	8
Cardiac/renal	4	0
Cardiac/cerebrovascular	0	3
Renal	33	4
Renal/cerebrovascular	3	0
Cerebrovascular	5	5
Cancer	1	8
Vascular	2	2
Suicide	2	0
Lung	0	2
Gastric	0	1
Unknown	5	5

cardiac disease is increasing. This pattern probably reflects improvements in the management of renal disease in patients with Fabry disease. These findings contribute to delineate the clinical profile of a multiorgan disease (Table **5**).

ANDERSON-FABRY DISEASE AS A MULTIORGAN DISEASE

AFD has long been regarded as an adult disease with most, if not all, affected males developing a "classic" phenotype. Later on, the sub-classifications "cardiac variant" [5,6] and "renal variant"[7] were introduced for patients with predominant or exclusive cardiac or renal involvement, respectively. Female heterozygotes were erroneously described as "carriers of the defective gene" more or less safeguarded against developing of disease manifestations and symptoms. However, evolving knowledge about the natural course of disease suggests that it is more appropriate to describe FD as a disease with a wide spectrum of heterogeneously progressive clinical phenotypes.

This spectrum ranges from the "classic" severe phenotype in males to a seemingly asymptomatic disease course occasionally observed in females, with a variety of clinical presentations [8] (Table **6**). Indeed, most female heterozygotes develop symptoms due to yet undetermined mechanisms [9,10] and a high percentage of females develops vital organ involvement including the kidneys, heart and/or brain about a decade later than males.

A number of Fabry cohort studies and post-marketing registry studies have been reported in the literature, describing the natural history of FD in males [11,12], females [13,14,15,16]. These studies have shown that despite residual enzyme activity, FD females do develop signs and symptoms of FD, such as left ventricular hypertrophy, proteinuria, stroke and, to a lesser extent, renal failure. The prevalence of acroparesthesia, a well known symptom of Fabry disease, ranges from 23 to 90% of the patients in the different cohort studies. These studies also reported a high prevalence of other non-specific complaints such as fatigue, palpitations, chest pain, abdominal pain and diarrhoea in FD females. As all of these symptoms are non-specific, care should be taken to attribute them directly to FD, especially as these studies lack control groups. At the outpatient clinic, Fabry females sometimes report symptoms or complaints of which both patient and the treating physician are unsure whether that complaint is part of the clinical spectrum of FD. The only way to appreciate whether certain complaints, aspecific or not, are part of the spectrum of FD is to study their prevalence in comparison with an appropriate control group. Bouwman *et al*. in a recent study [17] explore the prevalence of signs and symptoms in FD females in comparison to a control group. In this study FD females and age-matched controls were approached to complete a questionnaire. This questionnaire was developed by the Dutch Fabry patient organization (Fabry Support en Informatie Groep Nederland, FSIGN) with input from Fabry expert-physicians from the AMC. Authors compared the prevalence of symptoms using Pearson's chi-square test. Bonferroni correction was used to correct for multiple comparisons. A total of 63 heterozygotes and 52 controls completed the questionnaire. Many symptoms were also common in controls. In addition to acroparesthesia - fatigue, palpitations, dizziness, proteinuria during pregnancy, libido loss and joint pain are more prevalent in FD females as compared to a control group. Although, these symptoms are present in a significant proportion of normal controls they deserve further attention by treating physicians to better understand their significance, treatment and relationship with FD (Table **6**).

Another study with regard of type of multiorgan involvement according symptomatology in patients with AFD disease has been conducted by Wilcox *et al*. on the basis of Fabry Registry [18]. The Fabry Registry is a global clinical effort to collect longitudinal data on AFD. In the past, most "carrier" females were usually thought to be clinically unaffected. A systematic effort has been made to enroll all FD females, regardless of symptomology. Of the 1077 enrolled females in the Registry, 69.4% had symptoms and signs of FD. The median age at symptom onset among females was 13 years, and even though 84.1% had a positive family history, the diagnosis was not made until a median age of 31 years. Twenty percent experienced major cerebrovascular, cardiac, or renal events, at a median age of 46 years. Among adult females with estimated glomerular filtration rate (eGFR) data (N=638), 62.5% had an eGFR <90 ml/min/1.73 m^2 and 19.0% had eGFR <60 ml/min/1.73 m^2. Proteinuria 300 mg/day was present in 39.0% of females, and 22.2% had >1 gram/day. Quality of life (QoL), as measured by the SF-36((R)) survey, was impaired at a later age than in males, but both genders experience significantly impaired QoL from the third decade of life onward. Findings of this study have showed that females with AFD have a significant risk for major organ involvement and decreased QoL.

ORGAN INVOLVEMENT

Fabry Nephropathy

Renal failure is a serious complication of this disease. Fabry nephropathy lesions are present and progress in childhood while the disease commonly remains silent by routine clinical measures. Early and timely diagnosis of Fabry nephropathy is crucial since late initiation of enzyme replacement therapy may not halt progressive renal dysfunction. This may be challenging due to difficulties in diagnosis of Fabry disease in children and absence of a sensitive non-invasive biomarker of early Fabry nephropathy. Accurate measurement of glomerular filtration rate and regular assessment for proteinuria and microalbuminuria are useful, though not sensitive enough to detect early lesions in the kidney. Recent studies support the value of renal biopsy in providing relevant histological information to kidney function and prognosis, and renal biopsy could potentially be used to guide treatment decisions in young Fabry patients. This review aims to provide an update of the current understanding, challenges, and needs to better approach renal complications of Fabry disease in children. Progressive cellular accumulation of glycolipids starts early in life and, if untreated, eventu-

Table 5.

	Frequency		Age at symptom onset (years)	
	Proportion of population (%)	**Number of patients**	**Mean ± SD**	**Number of patients**
Cerebrovascular	20.0	75	33.5 ± 14.5	52
Stroke	8.5	32	39.4 ± 12.1	26
TIA(transient ischaemic attack)	8.8	33	32.4 ± 14.5	23
Neurological	82.9	311	16.3 ± 15.7	180
Pain attacks	69.3	260	13.6 ± 13.6	188
Chronic pain	49.9	187	17.2 ± 15.9	128
Cardiac	61.1	229	32.9 ± 14.5	141
Chest pain	19.5	73	38.4 ± 12.4	59
Palpitations	20.3	76	35.7 ± 12.8	51
Left ventricular hypertrophy	40.3	151	39.4 ± 11.5	92
Renal/urinary	57.3	215	33.9 ± 13.1	143
Proteinuria	45.9	172	33.2 ± 12.4	113
Haemodialysis	9.6	36	39.6 ± 13.2	32
Peritoneal dialysis	2.7	10	42.7 ± 7.1	8
Transplantation	8.8	33	37.2 ± 11.6	28
Gastrointestinal	56.3	211	23.3 ± 16.2	138
Diarrhoea	36.3	136	23.9 ± 16.9	89
Abdominal pain	41.1	154	22.4 ± 15.5	117
Constipation	13.6	51	23.3 ± 17.5	38
Auditory	56.3	211	29.9 ± 14.6	129
Tinnitus	31.7	119	28.1 ± 13.7	87
Vertigo	29.1	109	29.6 ± 14.4	77
Sudden deafness	6.1	23	30.8 ± 14.5	15
Ophthalmological	62.1	233	28.4 ± 14.7	151
Cornea verticillata	46.1	173	28.4 ± 14.0	120
Tortuous vessels	26.4	99	31.9 ± 13.1	67
Posterior subcapsular cataract	7.2	27	31.4 ± 16.0	16
Dermatological	70.1	263	18.7 ± 14.7	147
Angiokeratoma	65.9	247	18.7 ± 13.9	139
Telangiectasia	22.1	83	26.1 ± 16.6	46

Table 6. Prevalence of Symptoms in FD Females and Controls

	Fabry	Control	OR (95% CI)	P value
Cardiopulmonary symptoms				
Palpitations	29/61(48%)	7/53(13%)	5.96(2.32-15.3)	≤0.001
Skipped heartbeat	20/60(33%)	6/53(11%)	3.92(1.43-10.7)	0.006
Chest pain	18/62(29%)	5/51(10%)	3.76(1.29-11.0)	0.01
Shortness of breath	30/62(48%)	10/53(19%)	4.03(1.72-9.43)	0.01
Gastrointestinal symptoms				
Regurgitation	23/61(38%)	16/52(31%)	1.36(0.62-2.98)	0.44
Reflux	18/62(29%)	11/52(21%)	1.52(0.64-3.61)	0.34
Nausea	27/62(44%)	11/53(21%)	2.95(1.28-6.77)	0.01
Swallowing difficulties	19/61(31%)	6/53(11%)	3.54(1.29-9.71)	0.001
Hiccup	25/58(43%)	18/51(35%)	1.39(0.64-3.01)	0.40
Abdominal pain	37/62(60%)	16/53(30%)	3.42(1.58-7.43)	0.002
Diarrhea	25/61(41%)	10/53(19%)	2.99(1.27-7.03)	0.01
Constipation	21/61(34%)	5/53(9%)	5.04(1.74-14,6)	0.002
Flatulence	34/62(55%)	25/53(47%)	1.36(0.65-2.84)	0.41
Musculoskeletal symptoms				
Myalgia	35/62(57%)	19/53(36%)	2.32(1.09-4.93)	0.03
Muscle cramps	25/62(40%)	8/53(15%)	3.80(1.53-9.42)	0.003
Back ache	38/61(62%)	17/53(32%)	3.50(1.61-7.60)	0.001
Joint pain	36/62(58%)	13/52(25%)	4.15(1.86-9.29)	≤0.001
Pain in hands	36/62(58%)	6/53(11%)	10.85(4.04-20.1)	≤0.001
Pain in feet	24/61(39%)	5/53(9%)	6.23(2.17-17.9)	≤0.001
Neuropsychological symptoms				
Loss of strenght	28/62(45%)	8/53(15%)	4.63(1.88-11.4)	0.001
Numbness	8/61(13%)	8/53(15%)	0.85(0.30-2.44)	0.56
Fatique	55/62(89%)	30/53(57%)	6.02(2.32-15.7)	≤0.001
Dizziness	37/62(60%)	10/53(19%)	6.36(2.71-15.0)	≤0.001
Headache	36/62(60%)	24/53(45%)	1.67(0.80-3.59)	0.12
Depressed mood	34/62(55%)	20/51(39%)	1.88(0.89-3.99)	0.19
Urogenital symptoms				
Urinary incontinence	16/62(26%)	12/53(23%)	1.19(0.50-2.80)	0.69
Dysuria	3/62(4.8%)	1/53(1.9%)	2.64(0.27-26.2)	0.62
Premenstrual symptoms	26/40(65%)	20/34(29%)	1.30(0.51-3.34)	0.59
Menstrual symptoms	24/35(67%)	23/31(74%)	0.76(0.26-2.22)	0.42
Menarche at 12-15 years	44/62(71%)	36/53(68%9	NA	0.72
Fertility problems	5/40(13%)	1/39(2.6%)	5.43(0.60-48.8)	0.20

(Table 6) Contd….

	Fabry	Control	OR (95% CI)	P value
Loss of libido	27/45(60%)	9/31(23%)	3.67(1.38-9.75)	<0.001
Dermatological symptoms				
Dry skin	42/61(68%)	25/53(47%)	2.48(1.15-5.32)	0.03
Acne	10/61(16%)	6/53(11%)	1.54(0.52-4.55)	0.44
Eczema	11/62(18%)	7/53(13%)	1.42(0.51-3.96)	0.50
Vitiligo	6/62(10%)	5/53(9%)	1.03(0.21-3.58)	0.97
Urticaria	3/61(4.9%)	2/52(3.8%)	1.29(0.21-8.05)	1.00
Other symptoms				
Hypohidrosis	17/59(29%)	5/51(10%)	3.72(1.26-11.0)	0.01
Spontaneous tear in molar	13/60(22%)	10/49(20%)	1.08(0.43-2.73)	0.87
Allergy	10/62(16%)	9/53(17%)	0.94(0.35-2.52)	0.90
Snoring	20/62(32%)	31/53(56%)	0.34(0.16-0.73)	0.30
Gum disease	16/62(26%)	6/53(11%)	2.72(0.98-7.58)	0.05

ally leads to organ failure and premature death. The Fabry nephropathy is characterized by initial proteinuria in the second to third decades of life, and development of structural changes including glomerular sclerosis, tubular atrophy, and interstitial fibrosis. Progressive kidney failure develops at a comparable rate as in diabetic nephropathy. First signs of kidney damage may arise in childhood, prior to first signs of overt renal dysfunction underscoring the key importance of early recognition and diagnosis. Globotriaosylceramide (Gb-3) deposition is probably the initiating factor of the disease pathogenesis and, with enzyme replacement therapy (ERT), clearance can be achieved in several cell types. However, some late-stage effects are not reversible. As there is growing evidence that renal outcomes are more directly related to the degree of fibrosis and scarring, preventing the development of these irreversible changes by early initiation of ERT may have the greatest impact on renal outcomes. Proteinuria should be rigorously monitored and aggressively treated with antiproteinuric therapy.

Accumulation in the kidney is responsible for progressive decline in renal function in male patients with the classical phenotype, resulting in renal failure in their third to fifth decades of life. In a study by Valbuena *et al.* [19] GLA knockout mice ("Fabry mice") were generated as an animal model for Fabry disease but, as they do not manifest progressive chronic kidney disease (CKD), their relevance as a model for human Fabry nephropathy is uncertain. Authors evaluated the histological alterations in the kidneys of Fabry mice at different ages, as contrasted to those observed in wild-type mice. Furthermore, authors compared the renal histological alterations of Fabry mice to the kidney pathology reported in patients with Fabry disease at comparable age ranges and across different CKD stages, using a scoring system that has been developed for Fabry nephropathy. Fabry mice are phenotypically different from wild-type mice, displaying progressive age-related accumulation of glycosphingolipids in all types of renal cells. There were no statistically significant differences between Fabry mice and Fabry patients in the prevalence of glycosphingolipid storage per renal cell type with the exceptions of mesangial (higher in humans) and proximal tubular cells (higher in mice). However, Fabry mice lack the nonspecific histological glomerulosclerotic and interstitial fibrotic renal lesions that best correlate with progressive CKD in Fabry patients, and do not develop large podocyte inclusions. Findings of this study postulate that the elucidation of the mechanisms underlying these species differences, may contribute important clues to a better understanding of the pathogenesis of Fabry nephropathy.

Accumulation of Gb3 occurs mainly in the epithelial cells of Henle's loop and distal tubule, inducing early impairment in renal concentrating ability; involvement of the proximal tubule induces Fanconi syndrome. All types of glomerular cells are involved, especially podocytes, and glomerular proteinuria may occur at a young age. The evolution of renal Fabry disease is characterized by progressive deterioration of renal function to end-stage renal failure (ESRF). Ultrastructural study of kidney biopsies reveals typical bodies in the cytoplasm of all types of renal cells, characterized by concentric lamellation of clear and dark layers with a periodicity of 35-50 [20] (Fig. **3**).

In Anderson-Fabry disease the enzymatic defect, transmitted by an X-linked recessive gene, leads to the accumulation of neutral glycosphingolipids with terminal alpha-galactosyl residues in the plasma and in the lysosomes of endothelial, perithelial, and smooth muscle cells of the cardiovascular-renal system and, to a lesser extent, in reticuloendothelial, myocardial and connective tissue cells. Epithelial cells in the kidney, cornea, and other tissues contain the lysosomal depositions, as do the ganglia and perineural cells of the autonomic nervous system. The major accumulated substrate is globotriaosylceramide [galactosyl-(alpha 1----4)-galactosyl-(beta 1----4)-glucosyl-(beta 1----1')-ceramide]; another substrate, galabiosylceramide [galactosyl-(alpha 1----4)-galactosyl-(beta 1----1')-ceramide] is deposited primarily in renal lysosomes. The clinical manifestations of Fabry disease are the sequelae of the anatomical and physiologic alterations produced by progressive glycosphingolipid deposition. Renal manifestations are observed relatively early in the course of the disease. Renal biopsy specimens reveal evidence of diffuse intracytoplasmic glycosphingolipid accumulation, mainly affecting podocytes and epithelial cells of distal tubules, which are strikingly enlarged and vacuolated. On electron microscopy the deposits appear as typical osmiophilic inclusion bodies in the cytoplasm of all kinds of renal cells, and show a characteristic 'onion skin' or 'zebra' appearance. These pathological features are also evident in heterozygous females. Deposits occur

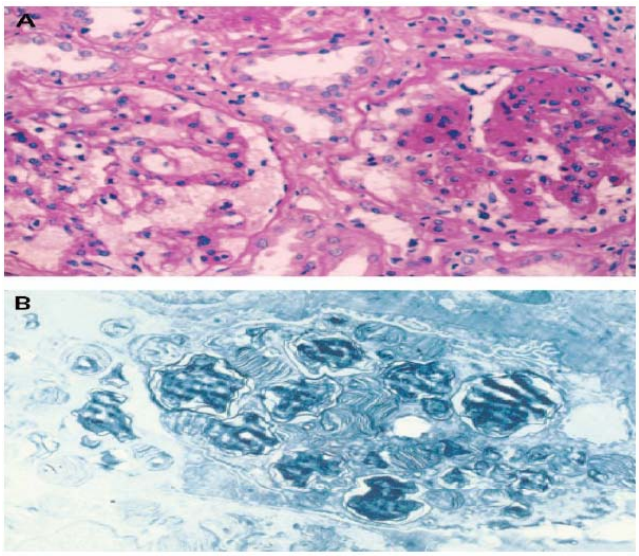


Fig. (3). A. Two glomeruli, 1 with marked vacuolation of glomerular epithelial cells (left) and 1 with moderately advanced sclerosis (right) (periodica-cid-Schiff stain). B. Electron photomicrograph of glomerular epithelial cells showing intralysosomal concentric lamellar membranous inclusions typical of Fabry disease. (from Joe T.R. Clarke, Narrative Review: Fabry Disease.Ann Intern Med 2007.

before the development of renal impairment. As patients age, the disease progresses in cells throughout the kidney, and is associated with increasing glycosphingolipid accumulation.

Clinical onset of the disease in hemizygous males usually occurs during childhood or adolescence, with periodic crises of severe pain in the extremities (acroparesthesias), the appearance of the vascular cutaneous lesions (angiokeratoma), hypohidrosis, and the characteristic corneal dystrophy (Fig. **4**). With increasing age, the major morbid symptoms of the disease result from the progressive infiltration of glycosphingolipid in the cardiovascular-renal system.

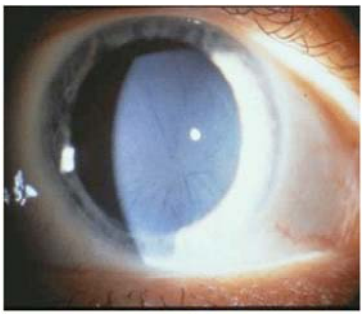


Fig. (4). Characteristic cornea verticillata in Fabry disease.

Death usually occurs from renal, cardiac, or cerebral complications of the vascular disease. Prior to the availability of treatment by renal transplantation or dialysis, the average age at death for affected males was about 40 years. Heterozygous females, who may exhibit the disease in an attenuated form, are most likely to have only corneal opacities. Previously, the diagnosis of affected hemizygous males and heterozygous females was based on clinical findings and the levels of alpha-galactosidase A activity in easily obtained sources, e.g., plasma and isolated lymphocytes or granulocytes. Because the gene encoding alpha-galactosidase A undergoes random X-inactivation, the expressed level of enzymatic activity in females heterozygous for the disease gene may vary significantly, thereby making accurate carrier detection difficult. With the advent of recombinant protein synthesis technology, enzyme replacement therapy has become a viable alternative to dialysis or renal transplantation, previously the only available treatment options for end-stage renal disease.

In a recent study [21] the pre- and post-treatment renal biopsies were analyzed from fifty-eight Fabry patients enrolled in a Phase 3 double-blind, randomized, placebo-controlled trial followed by a six-month, open label, extension study of the recombinant human enzyme, alpha-galactosidase A (r-hlphaGalA), administered IV at 1 mg/kg biweekly.

The purpose of this investigation was to detail the pathologic changes in glycosphingolipid distribution and the pattern of post-treatment clearance in the kidney. Baseline evaluations revealed Gb-3 accumulations in nearly all renal cell types including vascular endothelial cells, vascular smooth muscle cells, mesangial cells and interstitial cells, with particularly dense accumulations in podocytes and distal tubular epithelial cells. After 11 months of r-halphaGalA treatment there was complete clearance of glycolipid from the endothelium of all vasculature as well as from the mesangial cells of the glomerulus and interstitial cells of the cortex. Moderate clearance was noted from the smooth muscle cells of arterioles and small arteries. Podocytes (Fig. **5**) and distal tubular epithelium also demonstrated evidence for decreased Gb-3, although this clearance was more limited than that observed in other cell types. No evidence of immune complex disease was found by immunofluorescence despite circulating anti-r-halphaGalA IgG antibodies. These findings indicate a striking reversal of renal glycosphingolipid accumulation in the vasculature and in other renal cell types, and suggest that long-term treatment with r-halphaGalA may halt the progression of pathology and prevent renal failure in patients with Fabry disease (Tables **7**, **8**, **9**).

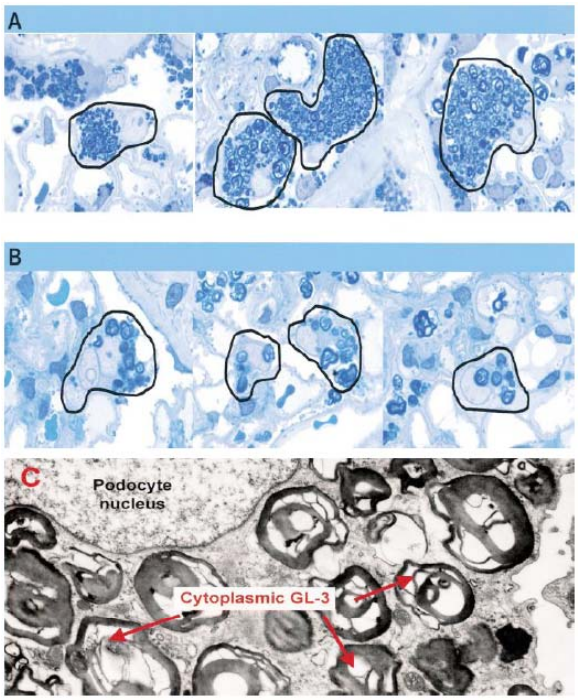


Fig. (5). Gb-3 is cleared from podocytes. (**A**) Baseline biopsy, pre-treatment. Gb-3appears as multiple, tight scroll-like figures that fill the cytoplasm of each podocyte. (**B**) Post-treatment. The scroll-like Gb-3 forms are looser and less numerous after treatment. Podocytes are outlined pre- and post-treatment for emphasis (magnification of **A** and **B**, ×100 objective). (**C**) Electron microscopic image demonstrating the lamellar ultrastructure of the Gb-3 accumulation in podocyte cytoplasm at baseline (magnification, 10,000).

Table 7. Scoring System for Gb-3 Accumulation/Clearance in Different Renal Cell Types

Cell/tissue	Score
Glomerular endothelial cells	0 = None or trace accumulation
Peritubular capillary endothelial cells	1 = Mild accumulation
Arterial/arteriolar endothelial cells Vascular smooth muscle cells	2 = Moderate accumulation
Podocytes[a]	3 = Severe accumulation
DCT/collecting ducts[a]	
Mesangial cells: Gb-3	
accumulation	0 = No lipid granules
Interstitial cells	1 = Minimal lipid granules
Mesangial matrix	2 = Numerous lipid granules
	0 = Normal mesangium
	1 = Mild expansion
	2 = Moderate expansion

[a] For the podocytes and distal convoluted tubules (DCT)/collecting ducts, an increase (+), a decrease (-) or no change (0) response was collected for all post-baseline scores by comparing the baseline biopsy to a 5 or 11 month biopsy. Therefore, there are no absolute baseline values for these cell types, only relative values at 5 months and 11 months that reflect a comparison to the baseline.

Table 8. Percentage of Patients Achieving Zero Scores[*] (Percentages of Patients with a 1-3 Point Score Reduction, Including all Zero Scores, are Shown in Parentheses)

		Score shift from baseline to 5 months			Score shift from baseline to 11 months		
Renal cells evaluated	TX group	Baseline	5 months	N	Baseline	11 months[a]	N
Peritubular capillary endothelial cells	Placebo	0%	0% (38%)	29	0%	100% (100%)	24
	A-gal	3%	69% (86%)	29	0%	92% (96%)	25
Glomerular capillary endothelial cells	Placebo	0%	0% (31%)	16	0%	100% (100%)	21
	A-gal	0%	100% (100%)	19	0%	100% (100%)	17
Mesangial cells	Placebo	0%	0% (19%)	16	0%	90% (100%)	21
	A-gal	0%	100% (100%)	19	0%	100% (100%)	17
Interstitial cells	Placebo	0%	0% (0%)	24	0%	78% (91%)	24
	A-gal	4%	71% (100%)	24	4%	100% (100%)	24
Arterial/arteriolar endothelial cells	Placebo	0%	0% (19%)	22	0%	87% (100%)	22
	A-gal	10%	86% (100%)	21	9%	96% (100%)	22
Vascular smooth muscle cells	Placebo	0%	0% (10%)	22	0%	0% (86%)	22
	A-gal	0%	10% (86%)	21	0%	0% (81%)	21

The clearance of Gb-3 from multiple renal cell types was determined before and after enzyme replacement therapy. There were two groups of patients: (1) the placebo group received placebo for the first 5 months and then α-galactosidase A for the remaining 6 months and (2) the A-gal group which received α-galactosidase A for the entire 11 months. The percentage of patients who attained a zero score at each biopsy time point is displayed and the percentage of all patients who demonstrated a reduction in score, including all zeros, is in parentheses. Values are presented for both the placebo and treatment groups. For statistical purposes, it was necessary for each patient to have both a baseline score and a shift score (5 months or 11 months) for each cell type, in order to be included in this data set. Two baseline columns are presented; the "N" designates the number of paired scores available for each cell type (paired scores meaning baseline and 5 months, or baseline and 11 months). This permitted calculation of the statistical significance of the change in score from either baseline to 5 months, or from baseline to 11 months, when comparing the placebo and treated groups. This distinction was necessary because (1) some patients had a poorly preserved or missed biopsy at one of the time points, and (2) some biopsy specimens did not contain glomeruli and therefore those cells could not be evaluated at all time points.

Table 9 Percentage of Patients Achieving a Reduction in Gb-3 Relative to Baseline*

		5 months		11 months[a]	
	TX group	**%**	**N**	**%**	**N**
Tubular epithelium	Placebo	4%	24	78%	24
	A-gal	25%	24	50%	24
Podocytes	Placebo	0%	16	23%	22
	A-gal	5%	19	18%	17

* Tubular epithelium and podocyte clearance were expressed as the percentage of patients attaining a relative reduction in the 5 month and 11 month biopsy. An increase (+), a decrease (-) or no change (0) response was collected for all post baseline scores by comparing the baseline biopsy to a 5 or 11 month biopsy. Therefore, there are no absolute baseline values for these cell types, only relative values at 5 months and 11 months that reflect a comparison to the baseline. A-gal represents patients treated with the recombinant human enzyme, -galactosidase A.

Furthermore, to determine the long-term safety and efficacy of recombinant human alpha-galactosidase A, an open-label, phase III extension study [22] was conducted, involving 58 patients who had classic Fabry disease and completed a 20-wk, double-blind, randomized, placebo-controlled, phase III study of agalsidase beta and were transitioned to an extension trial to receive biweekly 1 mg/kg agalsidase beta for up to an additional 54 mo. Gb-3 accumulation was evaluated in the capillary endothelia of the skin, kidney, and heart. Renal function was assessed (Figs. **6**, **7**). By month 54, all patients with optional kidney biopsies (n=8) maintained complete Gb-3 clearance in renal capillary endothelial cells and multiple cell types. Continued, complete clearance of skin (thirty-one of thirty-six) and heart (six of eight) capillary endothelium was demonstrated.

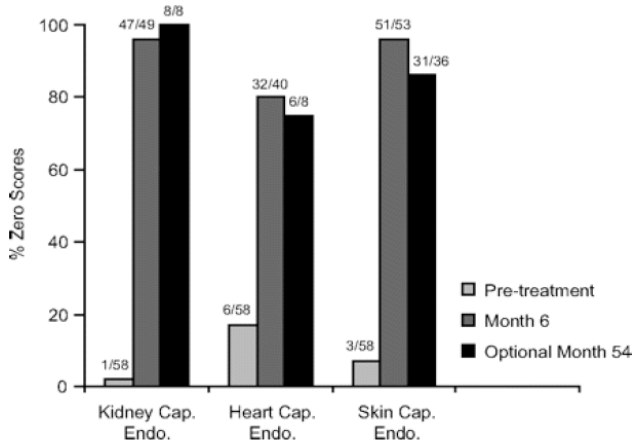


Fig. (6). Globotriaosylceramide (Gb-3) clearance from kidney, heart, and skin capillary endothelium in all patients. Histologic specimens that were obtained at pretreatment, month 6, and month 54 (optional for kidney and heart) were evaluated for the presence of Gb-3. Results show that for the majority of patients, initial Gb-3 clearance (score of 0) seen at month 6 was maintained during the 54-mo treatment period. Numbers above bar graphs represent the number of patients with zero scores out of the total number evaluated. cap. endo., capillary endothelium.

Mean plasma Gb-3 levels remained decreased in the normal range. Median serum creatinine and estimated GFR remained stable (normal) in patients with renal data at month 54 (n=41). Six patients had renal disease progression; most (four of six) were older than 40 yr and had significant proteinuria at baseline and evidence of sclerotic glomeruli pretreatment. Adverse events were generally mild and unrelated to treatment. The most common treatment-related adverse events were infusion-associated reactions, which decreased

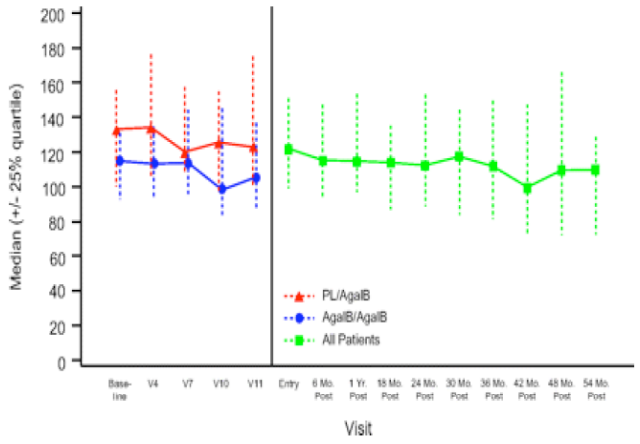


Fig. (7). Median estimated GFR (eGFR; ml/min per 1.73 m^2) over time. Patients in the "as treated" population maintained a stable median eGFR during the 54-mo treatment period, which was observed in both the placebo/agalsidase β and the agalsidase β/agalsidase β treatment groups (left) as well as in the overall, combined population (right).

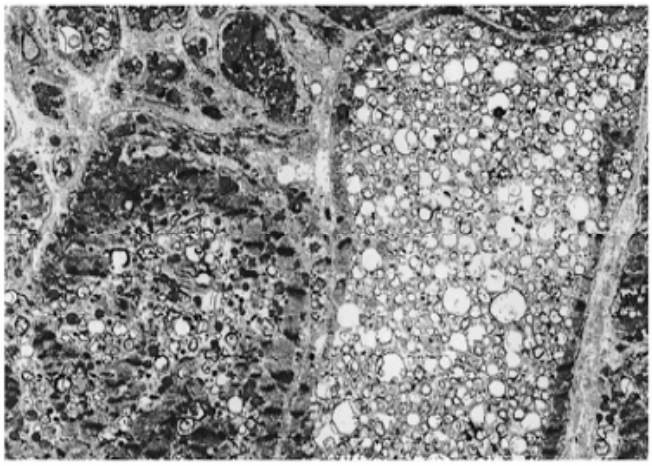


Fig. (8). Electron micrographs of a typical heart specimen of a AFD related cardiomyopathy showing the completely destroyed myocardial architecture. Of note are the multilamellar myelin bodies of the Gb-3 deposits and the additional vacuolization of the myocardium(Courtesy of Prof. Kuhn, Bielefeld)

over time. Long-term agalsidase beta therapy stabilizes renal function in patients without renal involvement at baseline, maintains

reduction of plasma Gb-3, and sustains Gb-3 clearance in capillary endothelial cells and multiple renal cell types.

The enzyme defect on the basis of AFD leads to progressive accumulation of glycosphingolipids (GL) in all kinds of cells, tissues, organs, and body fluids. The clinical manifestations are very protean, the residual activity of alpha-Gal-A and/or different gene mutations might explain different phenotypes, but as yet these concepts have not been proven. Usually, patients with AFD show 3 clinical phases, more evident in men than in heterozygous women. The first phase (childhood and adolescence) is characterized by myalgia, arthralgia, acroparesthesia, fever, cutaneous angiokeratomas, and corneal opacities. The second phase is characterized mainly by renal involvement. In the third phase, severe renal impairment and involvement of cerebrovascular and cardiovascular systems are present. The progression to end-stage renal disease (ESRD) is common in hemizygous males (3rd-5th decade of life); usually, death occurs because of cerebral and/or cardiovascular complications in patients undergoing chronic dialysis therapies. The survival of patients with AFD in dialysis is better than in diabetic patients, but it clearly is decreased compared with uremic patients with other nephropathies, despite a lower mean age of uremia (50 versus 60 y). The outcome of kidney transplantation is similar to that found in other patients with ESRD, despite controversial issues published in the past. The use of a kidney donor with normal alpha-Gal-A activity in the control of the metabolic systemic disease is unproven. The recurrence of GL deposits in the kidney graft has been documented rarely. The definitive treatment for AFD is enzyme replacement therapy with purified alpha-Gal-A produced by a genetically engineered human cell line or Chinese hamster oocytes: relatively short-term studies have shown a significant treatment. In the evaluation of early Fabry nephropathy, clinical assessments lack sensitivity, potentially delaying initiation of ERT [23] highlighting the utility of kidney biopsies as part of the baseline assessment. A similar scoring strategy was used for lupus nephritis [24] and focal segmental glomerulosclerosis [25]: [26]. Some authors [27] evaluated disease-specific Gb-3 lesions and secondary effect on clinical outcome measures, alterations that occur in Fabry nephropathy [28] as well as any other form of CKD (e.g. arterial and glomerular sclerosis, and interstitial fibrosis). Interstitial fibrosis, global and segmental sclerosis and podocyte inclusions were robustly scored. The latter parameter showed the highest degree of inter-individual agreement. The semi-quantitative scoring of interstitial fibrosis showed similar reliability when compared to the morphometric assessment. Other lesions (e.g. parietal epithelial cell inclusions, interstitial inflammation) could not be reliably scored with the current approaches.

The development of a standardized scoring system of both disease-specific lesions, i.e. lipid deposition related, and general lesions of progression, i.e. fibrosis and sclerosis, showed a spectrum of histologic appearances even in early clinical stage of Fabry nephropathy. These findings support the role of kidney biopsy in the baseline evaluation of Fabry nephropathy, even with mild clinical disease. The scoring system will be useful for longitudinal assessment of prognosis and responses to therapy for Fabry nephropathy.

AFD Vasculopathy and Cardiomiopathy

Fabry disease is an X-linked lysosomal storage disorder caused by deficiency of the enzyme alpha-galactosidase A and characterized by a progressive left ventricular hypertrophy mimicking the clinical phenotype of hypertrophic cardiomyopathy.

Although clinical features of the disease are mostly displayed in affected males, female carriers may suffer from Fabry related complications as well. The principal clinical manifestations in Fabry disease consist of artery associated complications (such as cerebral disease and nephropathy), but the pathophysiology of this specific vasculopathy is unclear. Several studies indicate that the specific vascular lesions that are present in Fabry disease occur as a result of vascular dysfunction with major components being endothelial dysfunction, alterations in cerebral perfusion and a pro-thrombotic phenotype [29,30]**.** Possibly, other cardiovascular risk factors may contribute to enhanced athero-thrombogenesis and a worsening of arterial performance. Although some patients with Fabry disease may suffer from stroke by involvement of larger arteries, small-vessel disease causes cerebral complications and probably contributes to complications of the kidney and the heart [31,32,33]. Controversy exists whether the storage in the endothelial cells and the pro-thrombotic state are the origin of arterial damage or whether smooth muscle cell proliferation in the arterial media layer is the initiating step in the cascade that leads to Fabry vasculopathy [34,35]. Supraventricular and ventricular arrhythmias are commonly observed in older patients with cardiac hypertrophy and in the advanced stage of the disease [36,37]. A report [38] shows that ventricular arrhythmias in Fabry disease are caused by infiltration of cardiac conduction tissue that can be privileged with respect to working cardiomyocytes giving rise to occult ventricular arrhythmias and sudden death even in young people.

Fabry cardiac involvement has several clinical manifestations (Table **10**): concentric left ventricular hypertrophy without left ventricular dilation and severe loss of left ventricular systolic function, mitral and aortic valvulopathy, disorders of the atrioventricular conduction or repolarization, and compromised diastolic function. Differentiating Fabry disease from similar conditions is often quite straightforward, e.g., cardiac amyloidosis is often associated with low electrocardiographic voltages, and systemic symptoms are usually associated with hemochromatosis and sarcoidosis. However, sometimes second-level (genetic analysis, alpha-galactosidase levels) or invasive investigations, which can include endomyocardial biopsy, are required (Fig. **8**).

Diagnostic imaging techniques have been described, but they lack specificity. Echocardiographic imaging with tissue Doppler analysis and/or strain rate analysis can allow diagnosis of Fabry disease even before left ventricular hypertrophy becomes apparent. This review illustrates the techniques for staging cardiac involvement and damage in Fabry disease and for the long-term follow-up of Fabry patients with or without cardiac involvement. Careful cardiac monitoring is especially important in elderly female carriers, who often develop renal disorders and/or left ventricular hypertrophy as the only manifestations of their late Fabry disease. In some clinical series, Fabry disease was diagnosed in 12% of women with adult-onset hypertrophic cardiomyopathy. Cardiological problems and outcomes of enzyme replacement therapy, associated with or without other cardiological treatments, are also discussed.

From previous studies, it is known that the morphological hallmarks of Fabry cardiomyopathy are LV hypertrophy and replacement fibrosis, which are both consistent with the finding of reduced regional LV function. In male patients, the LV starts to hypertrophy during adolescence; this is accompanied by reduced longitudinal function. The hypertrophy in these male patients progresses quite fast and finally leads to replacement fibrosis. In contrast, the fixed linkage of hypertrophy and fibrosis seemed to be not applicable for female patients with Fabry disease, although in general the same hallmarks (i.e., hypertrophy, reduced regional myocardial function, and replacement fibrosis) can be detected in the hearts of most female patients with Fabry cardiomyopathy. Thus, it seems that in this cohort, the progression toward severe hypertrophy in female patients was prolonged because of the residual activity of alpha galactosidase A (caused by the random inactivation of the mutated or non-mutated X chromosome). This is in concordance with previous studies done by Goldman *et al.* [39] and Linhart *et al.* [40] showing that LV hypertrophy is more common in male than in female patients. However, despite the delayed development of hypertrophy, fibrosis seems to progress continuously. Niemann *et al.* [41] hypothesized that Fabry cardiomyopathy in female patients might

Table 10. Cardiac Investigations. [From C. Golfomitsos *et al.* Fabry Disease. March 2012 Br J Cardiol 2012]

Electrocardiogram	Left ventricular hypertrophy Isolated repolarisation abnormalities (ST-T wave changes in leads I, II, aVL and V4–6) in absence of other causes such as hypertension, aortic stenosis Conduction abnormalities: short PR interval, 1, 2 or 3 degree heart block, bundle branch block, increased QRS voltage
Echocardiogram	Increased left ventricular mass (in patients with concentric remodelling or hypertrophy) Normal LV mass <134 g/m² for men and <110 g/m² in females Increased left ventricular wall thickness (13 mm in any segment) Left atrial enlargement Valvular thickening/ insufficiency Systolic impairment: regional wall motion abnormality or reduction in left ventricular ejection fraction (<50%) Diastolic dysfunction: using age corrected Doppler assessment Increased LV filling pressure on Doppler
24-hour electrocardiogram	Bradyarrhythmia Atrial arrhythmia Ventricular tachycardia
Exercise test	Positive
Angiography	Normal coronaries

differ substantially from that in male patients and sought to prove this hypothesis in a large cohort consisting of 104 patients with Fabry disease. Fabry cardiomyopathy in male patients is characterized by left ventricular (LV) hypertrophy, impaired myocardial function, and subsequent progressive myocardial fibrosis. In contrast, the occurrence of these 3 cardiomyopathic hallmarks in female patients remains unknown. In 104 patients (58 females, age 42 ± 16 years; 46 males, age 42 ± 13 years) with genetically proven Fabry disease, LV hypertrophy, regional myocardial deformation and myocardial fibrosis were assessed by standard echocardiography, strain rate imaging, and cardiac magnetic resonance (CMR) imaging-guided late enhancement (LE). In men, end-diastolic left ventricular wall thickness (LVWT) ranged from 6 to 19.5 mm [LV mass CMR 55 to 200 g/m(2)], and LE was never seen with LVWT <12 mm [LV mass <99 g/m(2)]. In contrast in female patients, LVWT ranged from 5 to 15.5 mm, LV mass ranged from 39 to 146 g/m(2), and LE was already detectable with an LVWT of 9 mm [LV mass 56 g/m(2)]. When LV mass was examined in CMR, LE was detected in 23% of the female patients without hypertrophy (n=9), whereas LE was never seen in male patients with normal LV mass. LE was always associated with low systolic strain rate, but the severity of impairment was independent of LVWT in female patients (lateral strain rate in patients with LV hypertrophy with LE -0.7 ± 0.2 s(-1); patients without LV hypertrophy with LE -0.8 ± 0.2 s(-1); p=0.45). In this study in contrast to male patients, the loss of myocardial function and the development of fibrosis do not necessarily require myocardial hypertrophy in female patients with Fabry disease. Thus, in contrast to actual recommendations, initial cardiac staging and monitoring should be based on LV hypertrophy and on replacement fibrosis in female patients with Fabry disease (Figs. **9**,**10**,**11**,**12**,**13**).

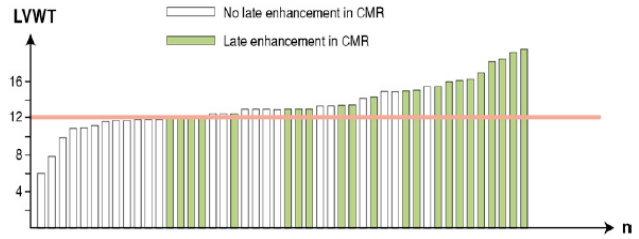


Fig. (9). Distribution of LE Depending on LVWT in Female Patients With Fabry Disease: Each bar symbolizes a single patient. Patients showing late enhancement (LE) are green bars. The patients are shown in the order of their left ventricular wall thickness (LVWT), the y-axis displaying the amount of end-diastolic LVWT. The pink line indicates the border toward LV hypertrophy. Note that some female patients were already LE positive (green bars) without being hypertrophic. CMR: cardiac magnetic resonance.

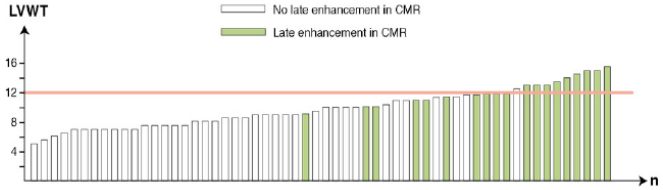


Fig. (10). Distribution of LE Depending on LVWT in Male Patients with Fabry Disease: Each bar symbolizes a single patient. Patients showing LE are green bars. The patients are shown in the order of their LVWT, the y-axis displaying the amount of end-diastolic LVWT. The pink line indicates the border toward LV hypertrophy. Note that male patients are only LE positive if they have an LVWT 12 mm and that male patients develop a larger amount of LV hypertrophy compared with female patients (Fig. **9**). Abbreviations as in (Fig. **9**).

Furthermore, in a study by Weidemann *et al.* [42], similar to other studies on morphological abnormalities in Fabry disease, the typical finding of LV hypertrophy was frequently detected. The increase of myocardial wall thickness appears to be an early marker indicating definite morphological change as a consequence of glycosphingolipid accumulation. The second typical morphological finding was late enhancement in the LV which is discussed as a marker for myocardial fibrosis. This study showed, in accordance with previous studies, that 50% of their patients had late enhancement predominantly in the LV inferolateral wall. The underlying mechanism for this mainly intramural pattern of late enhancement is unknown. An ischemic pathology is unlikely since previous studies excluded coronary artery disease in this group of patients and ischemic necrosis usually starts at the subendocardium, finally inducing a decrease in wall thickness in the chronic phase. It is known that LV work load is highest in the inferolateral wall. This might initiate development of myocardial fibrosis in these segments because a high work load may increase local wall stress.

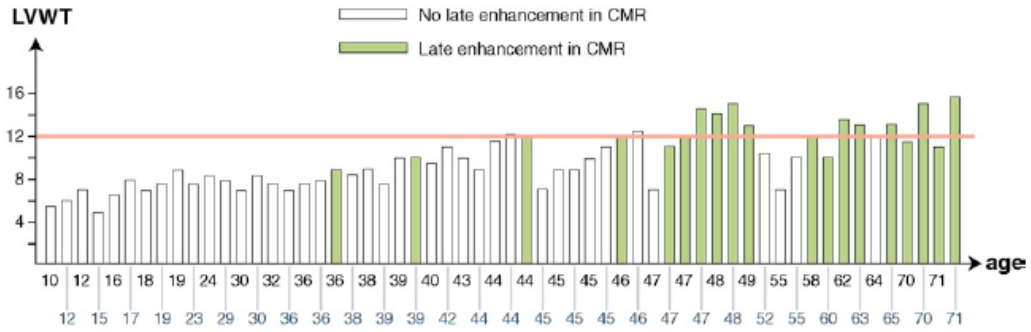


Fig. (11). Distribution of LE Depending on Age in Female Patients with Fabry Disease.
Each bar symbolizes a single patient. Patients showing LE are green bars. The patients are sorted in the order of their age. The y-axis displays the amount of end-diastolic LVWT. The pink line indicates the border toward LV hypertrophy. Note that some younger female patients who were nonhypertrophic already showed LE and some older patients did not show cardiac involvement at all. Abbreviations as in (Fig. **9**).

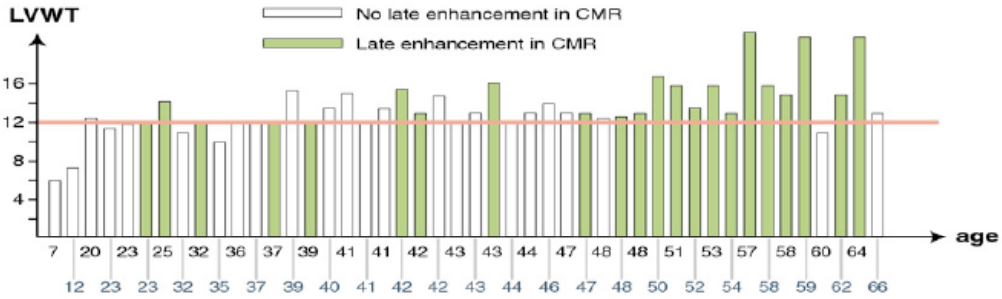


Fig. (12). Distribution of LE Depending on Age in Male Patients with Fabry Disease
Each bar symbolizes a single patient. Patients showing LE are green bars. The patients are sorted in the order of their age. The y-axis displays the amount of end-diastolic LVWT. The pink line indicates the border toward LV hypertrophy. Note that the onset of LE according to age in male patients was earlier than that in female patients. Abbreviations as in (Fig. **9**).

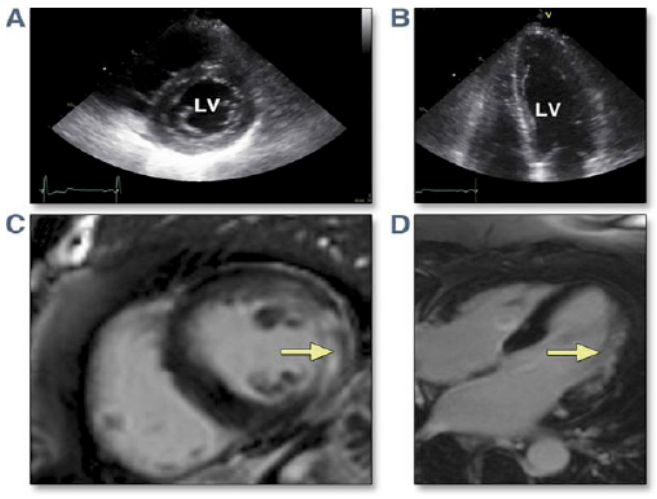


Fig. (13). Echocardiographic and CMR Images of a Female Patient without LV Hypertrophy but with LE (**A**) Short-axis echocardiographic view. (**B**) 4-chamber echocardiographic view. The LV cavity is marked with LV. The images clearly show that no LV hypertrophy is present. (**C**) Short-axis view of the same patient using the LE technique by CMR. (**D**) 4-chamber CMR view. The arrows indicate LE in thelateral wall.

In Anderson-Fabry disease (AFD), a progressive deposition of sphingolipids is reported in different organs. A recent study [43] applied 1H magnetic resonance spectroscopy (MRS) to investigate the myocardial lipid content. In patients proven AFD, 1H MRS of the heart was acquired in the same examination as routine cardiac cine and late enhancement MR imaging. Healthy volunteers (n = 11) without history of cardiac disease served as control (CTL). Myocardial triglycerides *in vivo* were quantified in 1H MRS. Left ventricular (LV) ejection fraction (EF) and late enhancement were assessed for the determination of LV systolic function, and onset or absence of myocardial fibrosis. Results: all 1H MRS revealed resonances for intramyocardial triglycerides. Clinical parameters, e.g. EF (PTS 64 ± 2 % vs. CTL 61 ± 1 %) were similar in PTS and CTL or showed a non-significant trend (LV mass). Apart from a single patient with elevated myocardial triglycerides, no significant impact of Fabry disease on the triglyceride/water resonance ratio (PTS 0.47 ± 0.11 vs. CTL 0.52 ± 0.11 %) was observed in this patient cohort. On this basis authors conclude that a comprehensive cardiac evaluation of morphology, function as well as metabolism in Fabry PTS with suspected cardiac involvement is feasible in a single examination. No significant effect of myocardial triglyceride deposition could be observed in patients. The remarkably high myocardial triglyceride content in one patient with advanced FD warrants further studies in PTS with an extended history of the disease.

AFD disease presents as a vascular disease, with cerebral, cardiac, and renal complications. Rombach *et al*. [44] measured carotid intima-media thickness (IMT), brachial flow-mediated dilation (FMD), pulse wave velocity, and advanced glycation end products in 57 classically affected patients (22 men and 35 women), 55 healthy matched controls (20 men and 35 women), and 10 atypical Fabry disease patients (5 men and 5 women). Most patients received enzyme replacement therapy. In classically affected male patients, brachial FMD was decreased (2.9% [95% CI, 0.8% to 7.9%] versus 5.9% [2.1% to 8.5%] in controls; P=0.01), and carotid

IMT was increased (0.67 mm [95% CI, 0.50-0.96 mm] versus 0.59 mm [95% CI, 0.40-0.76 mm] in controls; P=0.01). In women and atypical patients these vascular parameters were comparable with controls. Pulse wave velocity was not different; advanced glycation end products were only slightly increased in atypical patients. In classically affected women, a small increase in lysoGb3 was associated with an increase in IMT independent of age. In the classically affected men, all with increased IMT and high levels of plasma lysoGb3, lysoGb3 levels did not add to a higher IMT, suggestive of a ceiling effect. For FMD, elevated lysoGb3 levels (>7 nmol/L) contributed to a 2.9% lower FMD independent of age and sex (P=0.02). Increased carotid IMT and decreased brachial FMD occur in classic Fabry disease, which is associated with plasma lysoGb3 level independent of age and sex.

On this basis it's possible to underline that cardiovascular complications due to the accumulation of globotriaosylceramide in cardiac cells occur in almost all patients affected by Anderson-Fabry disease. Cardiac manifestations include left ventricular hypertrophy, mitral regurgitation, conduction disturbances and myocardial ischaemia. We recently reported a case of Fabry's disease diagnosed several years after the onset of early cardiac symptoms [45] (Figs. **14**, **15**,**16**).

CNS INVOLVEMENT

The neurological manifestations of Fabry disease include both peripheral nervous system and CNS involvement, with globotriaosylceramide accumulation found in Schwann cells and dorsal root ganglia together with deposits in CNS neurones. The main involvement of the CNS is attributable to cerebrovasculopathy, with an increased incidence of stroke. The abnormal neuronal accumulation of glycosphingolipid appears to have little clinical effect on the natural history of Fabry disease, with the possible exception of some reported mild cognitive abnormalities. The pathogenesis of Fabry vasculopathy remains poorly understood, but probably relates, in part, to abnormal functional control of the vessels, secondary to endothelial dysfunction as a consequence of α-galactosidase A deficiency (Fig. **17**).

Obstructive vasculopathy, either primarily due to accumulation of glycolipid or secondary to consequent inflammation and con-

Analisi	Valore attività enzimatica nmoli/h/ml	Valori di Riferimento nmoli/h/ml
Enzimatica	0,4 nmol/ml/h	Uomini < 1 soggetto Fabry; ≤ 3 sospetta inefficienza enzimatica; > 3 sano Donne (1) (1) nelle donne i valori enzimatici non sono indicativi e presuppongono comunque l'indagine genetica.
Genetica(2)	E' stata riscontrata la seguente mutazione nel gene *GLA:* **g.7446G>A che determina la sostituzione aminoacidica G183S**	

(2) l'errore diagnostico derivante dalle tecniche di biologia molecolare utilizzate è inferiore all'1.5 %.

Fig. (14). Enzymatic assay revealed a reduced plasma alpha galactosidase A activity: 0.4nmol/ml/h, which is a typical value for hemizygous Fabry patient. Genetic DNA sequence analysis detected the presence of a point mutation.

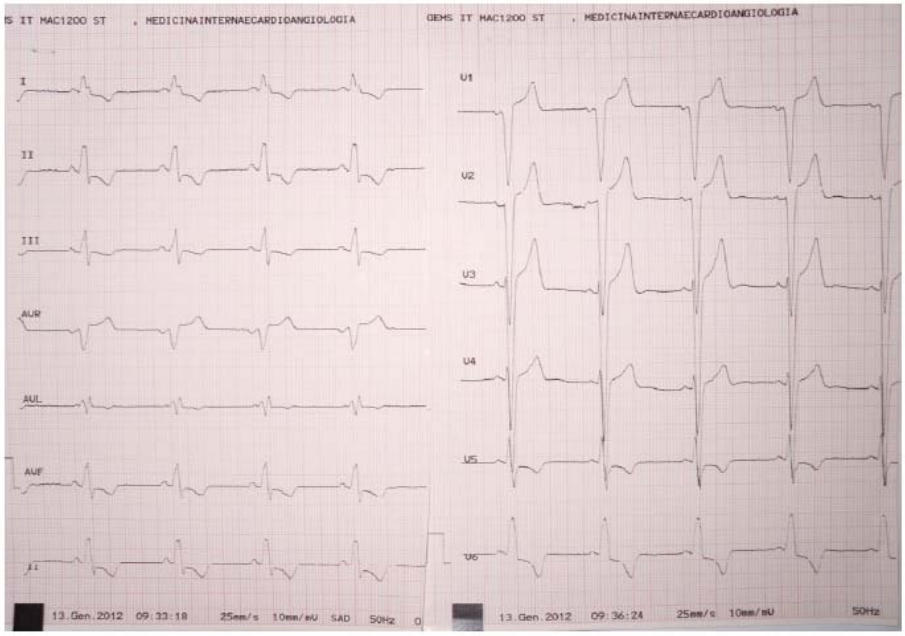


Fig. (15). ECG: showing electrical signs of left ventricular hypertrophy, reduced PR interval, LBBB, alterations in the ventricular repolarization phase in anterolateral leads, in a 55-year-oldmale patient with AFD.

Fig. (16). Echocardiography shows left ventricular hypertrophy, moderate-to-severe systolic dysfunction; left ventricular endocardium appears hyperechogenic.

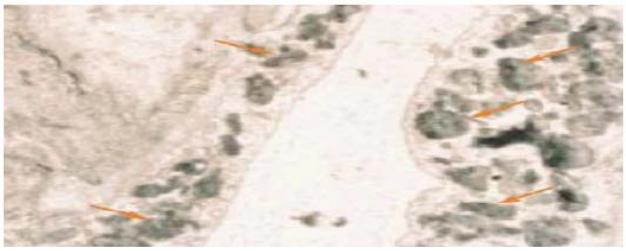


Fig. (17). Electron micrograph showing the vascular endothelium of a small vessel from a patient with Fabry disease. Progressive accumulation of substrate leads to ischemia and eventual vessel infarction. (From R.J. Desnick, MD, Phd)

founding vascular risk factors, may develop in response to abnormal endothelial and vessel wall function, similar in some respects to that observed with accumulation of cholesterol-laden lipids during atherosclerosis. Involvement of the peripheral nervous system affects mainly small Aδ and C fibres, and is probably causally related to the altered autonomic function and neuropathic pain found in Fabry disease. Other related neurological problems include hypohidrosis and other abnormalities associated with nervous system dysfunction.

A recent study [46] described CNS involvement in a group of Italian patients with AFD. Clinical and brain MRI data of 43 patients with AFD (25 men, 41.94+/-10.83 years old and 18 women, 52.48+/-17.50 years old) were analysed retrospectively. 17 male patients and 7 female patients were under treatment with enzyme replacement therapy (ERT). All 43 patients had signs or symptoms of AFD. 16 men (64%) and 13 women (72%) demonstrated CNS involvement, although with varying severity. Overall, 6 men and 5 women had suffered from cerebrovascular accidents with an age at onset of 33.64+/-13.65 years and 53.68+/-11.71 years, respectively. Brain MR images were abnormal in 16/25 men and in 13/16 women. During CNS monitoring, some patients receiving ERT (5/17 men and 2/6 women) demonstrated neurological deterioration, especially those who had presented with cerebrovascular disease already before starting ERT. This study demonstrated a high frequency of CNS involvement in homozygous and heterozygous AFD patients, often characterised by early age at onset and abnormal brain MRIs. At present, ERT is widely used; however, potential beneficent effects may be disguised by the progression of irreversible pathology in short-term follow-up. Therefore, primary and secondary prophylaxes of cerebrovascular disease are extremely important. Along with progressive renal and cardiac dysfunction, stroke is a major and often life-threatening burden of the disease. Cerebral vasculopathy, confirmed by neuropathological, neuroradiological, and functional studies, occurs commonly and leads to ischaemic cerebrovascular events at an early age. Fabry's disease is an X-linked disease and women have been regarded as only mildly affected carriers. However, research has shown a high prevalence of ischaemic stroke and transient ischaemic attacks, along with imaging evidence of CNS involvement, in female patients with the disease, which suggests that at least in a subgroup of clinically affected women the severity of CNS disease is comparable to that in men. These new findings should be replicated in larger samples. Brain structural changes and CNS involvement in the disease need to be monitored carefully in follow-up studies to broaden our knowledge of the course of neurobiological changes and to identify potential effects of enzyme-replacement therapy, which is already showing some benefit in cardiac and renal dysfunction in the disease. On this basis a diagnosis of Fabry's disease should always be considered in young patients who have had a stroke. Neutral glycosphingolipides (esp. Gb3) accumulate in lysosomes of several tissues, particularly in vascular endothelium and smooth muscle cells. Cerebral manifestations that might be mainly due to progressive cerebrovascular dysfunction, are one major and often life-threatening burden of the disease. We reviewed the present literature concerning brain structural alterations in FD and discussed the possibly relevant underlying pathophysiological aspects of these disturbances. Cerebrovascular events (TIA, stroke) occur in FD at a rather early age. In female FD patients who were considered to be less affected "carriers" for a long time, the prevalence of cerebrovascular events seems to be at last as high as in male patients. In structural imaging white matter lesions (WML) can be found frequently even in young FD patients. Different pathophysiological aspects of cerebral angiopathy and WML development are discussed against the background of current concepts (e.g. accumulation of Gb3 in vascular endothelium with consecutive cell proliferation and luminal stenosis, acceleration of focal intravasal pressure and disturbances of vascular auto-regulation). Pathological increase of pulvinar signal in T1-weighted MRI has also been described in FD. This finding was assumed to be caused by calcification as a consequence of disturbed local circulation. To enhance our knowledge about the relevant neurobiological processes the some authors propose a more sensitive and early detection of brain structural changes in FD. New brain structural MRI methods such as diffusion-tensor imaging could provide a pattern of ultrastructural changes even in young patients without visible WML. This strategy could be as well useful for quantification of possible effects of the enzyme replacement therapy on brain structural alterations in FD. Based on recent data a systematic FD-screening by measuring Gb3 in urine of young patients with cryptogenic stroke should be discussed. Basically in such cases AFD should be clinically considered.

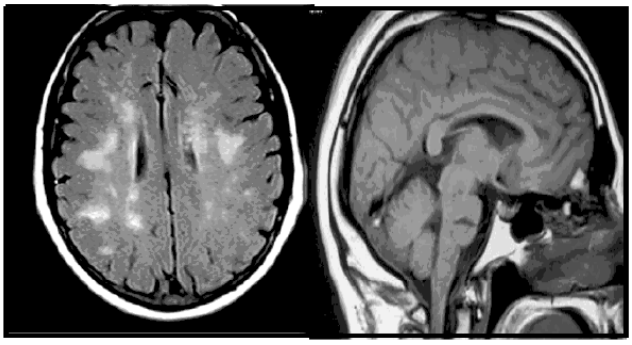
A study conducted by Marino *et al.* [47] assessed structural and metabolic brain changes in subjects affected by Fabry disease (FD) or carrying the disease mutation (Table **11**; Fig. **18**).

Previous MR studies have shown structural and metabolic brain abnormalities in FD patients. It is not clear, however, whether tissue damage can be seen in both the brains of hemizygous and heterozygous and whether quantitative MR metrics are useful to monitor disease evolution. Authors studied 4 males and 4 females with FD. Each subject underwent brain proton MRI/MR spectroscopic imaging (MRSI) examinations to obtain measures of total brain volumes, total brain lesion volumes, magnetization transfer ratios (MTr) in

Table 11. CNS Involvement: Clinical Features of the 43 Patients with AFD

	Male patients (n = 25)	Female patients (n = 18)
	Number of patients (%)	Number of patients (%)
Demographic characteristics		
Age (mean ± SD)	41.94 ± 10.83 years	52.48 ± 17.50 years
Range of age	21–62 years	19–74 years
Most common CNS manifestations		
A) Total no. of patients with cerebrovascular events	6 (20%)	5 (28%)
TIA	5 (20%)	3 (17%)
Stroke	6 (24%)	5 (28%)
Age at onset (mean ± SD)	33.64 ± 13.65 yrs	53.68 ± 11.71 yrs
Recurrence	6 (24%)	4 (22%)
First episode during ERT	0 (0%)	0 (0%)
B) Total number of patients with minor neurological manifestations	15 (60%)	12 (67%)
Diplopia	5 (20%)	3 (17%)
Migraine/recurrent headache	5 (20%)	5 (28%)
Vertigo	8 (32%)	2 (11%)
Hearing loss	9 (36%)	6 (33%)
Reported cognitive problems	3 (12%)	4 (22%)
Total percentage of patients *with* any kind of clinical CNS manifestation	16 (64%)	13 (72%)
Total percentage of patients *without* any kind of clinical CNS manifestation	9 (36%)	5 (28%)
Clinical outcome		
Rankin scale ≥2	9 (36%)	4 (22%)
EuroQol ≥3	4 (16%)	4 (22%)

CNS, central nervous system; ERT, enzyme replacement therapy; manifest., manifestations; No., number; SD, standard deviation; TIA, transitory ischaemic attack.



A B

Fig. (18). A: Brain MRI(Flair)of a 54 year-old female patient with AFD: multifocal silent leucoencephalopathy. **B**: Brain MRI(T1) of a 36 year-old male patient with AFD: lacunar infarction and cerebral atrophy

WM and central brain levels of N-acetylaspartate (NAA) to creatine (Cr). A second MR examination was performed in five subjects after 2 years. Focal WM lesions were found in 2 males and 1 female. The MTr values were always low in the WM lesions of FD subjects ($p < 0.001$) and also were low in the normal-appearing WM of 2 affected males. Total brain volumes were never decreased in FD subjects. Brain NAA/Cr values were significantly ($p = 0.005$) lower in FD subjects than in normal controls and correlated closely with Rankin scale measures ($r = -0.79$). On follow-up examinations, no significant MR changes were found. However, the small changes in NAA/Cr correlated closely with changes in Rankin scores ($r = -0.86$). Subtle structural and metabolic tissue damage can extend beyond WM lesions in FD subjects. Diffuse brain NAA/Cr decrease can be found in FD subjects in relation to the degree of their CNS involvement and its evolution over time.

Nevertheless in a recent clinical, neuropathological and neurochemical study [48] of a case of Anderson-Fabry's disease the neuropathological examination is dominated by severe alterations in the cerebral vessels due to glycolipid deposits on the walls, with reduction or occlusion of the lumen. This is correlated with secondary ischaemic foci scattered throughout the cortex as well as through the white matter. In addition, the cells of the cerebral cortex, thalamus, basal ganglia, amygdala, cerebellar and olivary nuclei show a marked accumulation of lipofuscin. Biochemical examination reveals a threefold increase in galactolipids due to the specific alpha-galactosidase deficiency. Cholesterol is reduced secondarily to ischaemic myelin damage. Glycosaminoglycans uronic acid is increased in cytosol and membrane-bound fractions which could be related to reactive gliosis. Glycoprotein sugars show a decrease in N-acetyl-neuraminic acid and fucose as well as an increase in hexosamines and hexoses in membrane-bound fraction, while in cytosol fraction all sugars are increased. This suggests that the alpha-galactosidase deficiency can alter not only the glycolipid but also the glycoprotein metabolism, resulting in a higher presence of hexosamines and hexoses-rich glycoproteins.

Furthermore, ischemic stroke is related to inflammation [49,50, 51,52] and arterial stiffness [53,54] and no study had addressed this relationship in patients with AF disease and cerebrovascular disease, so this topic could represent a possible future research line.

SKIN INVOLVEMENT

The diagnosis of Fabry disease is made in hemizygous males after the detection of the presence of angiokeratomas (Fig. **19A**, **B**), irregularities in sweating, edema, scant body hair, painful sensations, and of cardiovascular, gastrointestinal, renal, ophthalmologic, phlebologic, and respiratory involvement. A deficiency of alpha-gal A in serum, leukocytes, tears, tissue specimens, or cultured skin fibroblasts further supports the diagnosis in male patients. Since heterozygous women show angiokeratomas in only about 30% of

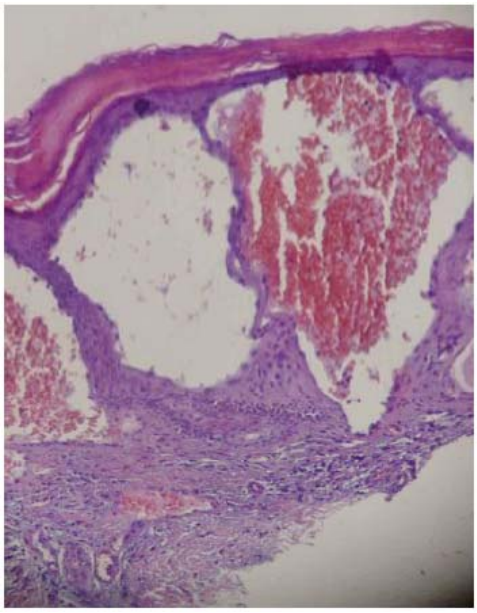


Fig. (19) A. Skin biopsy (light microscopy): histologically, the typical skin lesion is a small superficial angioma caused by cumulative damage of the vascular cells of the dermis with vessel dilation.Courtesy: Dr Juan M. Politei Buenos Aires, Argentina.

Fig. (19). B. Angiokeratoma in a 28-year-old male patient with AFD. Angiokeratomas are small, raised, dark-red spots that increase in number and size with age and can occur singly or in clusters. They are typically found on the lower back, buttocks, groin, flanks and upper thighs but their distribution may be restricted to a limited area, such as the umbilicus.

cases and may have alpha-gal A levels within normal range, genetic analysis is recommended.

When cutaneous and mucous glands are affected, restrictions may be required with regard to the time spent in a warm climate and the amount time spent working or on sporting activities, and may necessitate the use of topical and systemic antiperspirant agents, and topical application of artificial lacrimal fluid and saliva, respectively. For the future, new treatment modalities, including enzyme replacement therapy, substrate deprivation strategies, and gene therapy offer extraordinary options for the cutaneous and visceral lesions in patients with Fabry disease.

In Anderson-Fabry disease lysosomal deposits of ceramide trihexoside have been repeatedly documented in a wide range of tissues, including those found in angiokeratoma, the characteristic [55] cutaneous lesion which allowed the definition of Fabry disease. In a recent study authors investigated whether there was any difference in the amount of dermal lysosomal storage in males and females, thus accounting for the difference in clinical severity of both groups. For that purpose, with electron microscopy and quantitative methods, they studied the extent of lysosomal deposits in dermal fibroblasts of normal-appearing skin in six females and nine men, enzymatically and genetically proven as to have Fabry disease, and results were compared. These results indicate a statistically significant difference between the two groups regarding both the percentage of dermal fibroblasts bearing stored material, and the storage surface occupied in 100 fibroblasts per case.

In a study by Orteu *et al.* [56] authors ascertained the prevalence and nature of cutaneous manifestations in patients with Fabry disease and relate these to the severity of systemic manifestations of the disease. They have documented the dermatological features of this disease with reference to data from 714 patients (345 males, 369 females) registered on the Fabry Outcome Survey (FOS), a multicentre European database. Authors confirmed that the commonest disease manifestation is angiokeratoma. Overall, 78% of males and 50% of females had one or more dermatological abnormality, the commonest being angiokeratoma (66% males, 36% females), hypohidrosis (53% males, 28% females), teleangectasia (23% males, 9% females) and lymphoedema (16% males, 6% females). They demonstrate for the first time that the presence of cutaneous vascular lesions correlates with the severity of the systemic manifestations of the disease (pain, renal failure, cardiac disease, premature cerebrovascular disease) as assessed by a severity scoring system. Although the condition is X linked, there is a surprisingly high prevalence of abnormalities in females.

The FOS database is a useful epidemiological tool in establishing the variety and relevance of cutaneous manifestations in Fabry disease. The present study confirms that the presence of dermatological manifestations appears to be a marker of greater severity of systemic disease, which emphasizes the importance of the dermatological assessment of these patients.

AFD AS A MULTIORGAN DISEASE, POTENTIAL GENETIC AND CIRCULATING MARKERS OF MULTIORGAN INVOLVEMENT: CONCLUSIONS

Fabry disease is a rare lysosomal storage disorder caused by the deficient activity of the lysosomal enzyme alpha-galactosidase A. The resultant storage of undegraded glycolipids leads to the progressive development of potentially life-threatening manifestations affecting multiple organ systems in the body. The Mainz Severity Score Index (MSSI) (Table **12**), a scoring system for patients with Fabry disease has been proven to be representative in patients with 'classic' Fabry disease and may be useful for monitoring clinical improvement in patients receiving enzyme replacement therapy. The MSSI of patients with AFD was significantly higher than that of patients with other severe debilitating diseases. In a recent study by Whymbra *et al.* [57] the MSSI indicated that, although more men than women had symptoms classified as severe, overall, the median total severity scores were not significantly different between male and female patients. One year of ERT with agalsidase alfa led, in all patients, to a significant ($p < 0.001$) reduction in MSSI score (by a median of nine points).

Some authors conducted a study [58] that showed that the MSSI score may be a useful, specific measure for objectively assessing the severity of AFD and for monitoring ERT. Fabry disease is a multisystem disorder with phenotypic heterogeneity only partially explained by genotype. Elevated interleukin-6 (IL-6) plasma levels and C-reactive protein (CRP) serum levels are associated with increased risk and worse outcome of ischaemic events, a serious prognostic sign in Fabry disease. In this study 56 patients (34 hemizygous males, 22 females; 5 children) were studied. A

Table 12. The Mainz Severity Score Index (MSSI) and the Fabry Outcome Survey adaptation of the Mainz Severity Score Index (FOS-MSSI).

	General score					Neurological score			
Sign/symptom	**Rating**	**MSSI score**	**FOS-MSSI score**	**Adaptation to signs and symptoms**	**Sign/symptom**	**Rating**	**MSSI score**	**FOS-MSSI score**	**Adaptation to signs and symptoms**
Characteristic facial appearance	No	0	-		Tinnitus	No	0	0	
	Yes	1	-			Mild	1	1	Any
Angiokeratoma	None	0	0			Severe	2	-	
	Some	1	1.5	Any	Vertigo	No	0	0	
	Extensive	2	-			Mild	1	1	Any
Oedema	No	0	0			Severe	2	-	
	Yes	1	1		Acroparaesthesia	No	0	0	
Musculoskeletal	No	0	0			Occasional	3	4	Pain attacks
	Yes	1	1			Chronic	6	6	Chronic pain
Cornea verticillata	No	0	0		Fever pain crisis	No	0	-	
	Yes	1	1			Yes	2	-	
Diaphoresis	Normal	0	0		Cerebrovascular	No	0	0	
	Hypo/hyper	1	1			Ischaemic lesions (in MRI/CT)	1	3	
	Anhidrosis	2	2			TIA/migraine etc.	3	6	TIA, PRIND
Abdominal pain	No	0	0			Stroke	5	-	
	Yes	2	2		Psychiat-ric/psychosocial				
Diarrhoea/constipation	No	0	0		Depression	No	0	0	
	Yes	1	1			Yes	1	1	
Haemorrhoids	No	0	0		Fatigue	No	0	-	
	Yes	1	1			Yes	1	-	
Pulmonary	No	0	0		Reduced activity level	No	0	-	
	Yes	2	2	Breathing difficulties		Yes	1	-	
New York Heart	No	0	0						
Association (NYHA) classification*	Class I	1	-						
	Class II	2	2	Angina					
	Class III	3	-						
	Class IV	4	-						
Maximum score		18	13.5		Maximum score		20	15	

(Table 12) Contd….

	Cardiovascular score					Renal score			
Sign/symptom	**Rating**	**MSSI score**	**FOS-MSSI score**	**Adaptation to signs and symptoms**	**Sign/symptom**	**Rating**	**MSSI score**	**FOS-MSSI score**	**Adaptation to signs and symptoms**
Changes in cardiac muscle thickness	No	0	0		Evidence of renal dysfunction	No proteinuria	0	0	
	Thickening of wall/septum	1	-			Proteinuria	4	4	Haematuria/proteinuria
	LVH seen on ECG	6	6			Tubular dysfunction/low GFR or creatinine clearance	8	8	Renal failure
	Cardiomyopathy (< 15 mm)	8	10	Heart failure		End-stage renal failure (serum creatinine levels > 3.5 mg/dl)	12	-	
	Severe cardiomyopathy (> 15 mm)	12	-			Dialysis	18	18	Dialysis/transplantation
Valve insufficiency	No	0	0						
	Yes	1	1	Valve disease					
ECG abnormalities	No	0	0						
	Yes	2	2	Conduction abnormalities and arrhythmia					
Pacemaker	No	0	0						
	Yes	4	4	Surgery					
Hypertension	No	0	0						
	Yes	1	1						
Maximum score		20	18		Maximum score		18	18	

*Limitation on physical activity according to NYHA classification is as follows. Class I: none; ordinary physical activity does not cause undue fatigue, palpitation, dyspnoea or anginal pain, but echocardiography reveals heart involvement. Class II: slight; comfortable at rest, but ordinary physical activity results in fatigue, etc. Class III: marked; comfortable at rest, but less than ordinary physical activity causes fatigue, etc. Class IV: unable to carry out any physical activity without discomfort; symptoms of cardiac insufficiency or of anginal syndrome may be present even at rest and physical activity increases discomfort.

CT, computed tomography; ECG, electrocardiography; GFR, glomerular filtration rate; LVH, left ventricular hypertrophy; MRI, magnetic resonance imaging; PRIND, prolonged reversible ischaemic neurological deficit; TIA, transient ischaemic attack.

promoter polymorphism -174G > C of the IL-6 gene associated with serum IL-6 levels was compared with the Mainz Severity Score Index (MSSI) in patients with Fabry disease. CRP levels and polymorphism 1059 G > C were evaluated as markers of inflammation to ascertain the possibility of an inflammatory mechanism of IL-6. Nonparametric ANOVA, Fisher's exact, Bonferroni, and Hardy-Weinberg (HW) statistics were used.

Significant correlation existed between all sub-scores of the MSSI and IL-6 genotypes in females but only with three MSSI sub-scores for males. The IL-6 C/C genotype was significantly correlated with the neurological, general and total MSSI sub-scores, generally twofold higher. There were no statistically significant correlations with CRP levels/polymorphisms and MSSI sub-scores nor with IL-6 polymorphisms. CRP levels decreased after ERT in patients with IL-6 G/G or G/C genotypes but increased in patients with C/C ($p = 0.003$).

The prevalence of the IL-6 C allele significantly influences MSSI, i.e. clinical severity, especially in females. This is unrelated to IL-6 as a pro-inflammatory marker as demonstrated by lack of correlations with CRP levels and genotypes. IL-6 -174 polymorphic C allele may be a prognostic marker in Fabry disease, especially in females, may be a expression of the multiorgan disease and probably ERT-related treatment effects.

Another study [59] evaluated functional gene polymorphisms of key pro- and anti-inflammatory cytokines and to correlate them to a clinical score to assess the potential role of inflammation in

Fabry disease. Genotyping for IL-10[819C/T; -592C/A]; IL-1beta[+3954 C/T; -511C/T]; IL-1alpha[-889C/T]; and TNF-alpha[-308G/A] was performed in 76 patients and correlated with MSSI sub-scores and with enzyme (alpha-galactosidase A) levels. Fifty, normal, age- and sex-matched volunteers were also genotyped. Of 76 patients, 31 (41%) were males and 45 (59%) were females. There was no correlation between enzyme levels and any cytokine

levels. Statistically significant differences were found in prevalence of TNF-alpha [-308G/A] genotypes: 84% GG in patients versus 63% GG in controls ($p = 0.038$) and for IL-1alpha [-889C/T] genotypes: 94% CC in patients versus 21% CC in controls ($p < 0.001$). Statistically significant differences were found in the ratio between the two polymorphisms of IL-10 ($p < 0.0001$), between the two polymorphisms of IL-1beta ($p = 0.001$); between IL-1alpha [-889C/T] and IL-1beta [3954C/T] ($p = 0.002$); and between IL-10[-592C/T] and IL-1beta [3954C/T] ($p = 0.041$). Correlations between TNF-alpha [-308G/A] and both kidney and neurological MSSI subscores (both: $p = 0.06$) and between IL-10[-819C/T] and the MSSI neurological score ($p = 0.03$) were noted (Tables **13**, **14**,**15**).

The majority of patients with Fabry disease have therefore a profile of low TNF-alpha (increased frequency of GG genotype of the TNF-alpha polymorphism), high IL-10 production (preponderance of the C allele of the wild type or heterozygous state for the polymorphisms of IL-1, but simultaneously increased production of the pro-inflammatory cytokines IL-1beta and IL-1alpha usually associated with a preponderance of the C allele of the wild type or heterozygous state for the polymorphisms of IL-1beta and of IL-1 alpha. Authors speculated that sequence variations of important inflammatory genes of the interleukin inflammatory family are associated with differential effects in Fabry disease, and with increased sample size, haplotype blocks might be constructed.

Fabry syndrome is a genetic disease related to changes on the X chromosome. Its complex clinical presentation and diverse symptomatology is caused by deficient activity of lysosomal hydrolase alpha-galactosidase enzyme. Defect in the basic alpha-galactosidase molecule implies genetic change, which can be a predisposing factor for the development of atypical and typical forms of this genetic disease. Clinical manifestation and hemizygous symptomatology were the evidence of metabolic and genetic irregularity, typical clinical presentation of Fabry disease. Many authors report generalized vasculopathy as a basic characteristic of Fabry disease and a causative factor of multiorgan changes. Some authors indicate that persons with diagnosed asymmetric hypertrophy of the left ventricle have decreased alpha-galactosidase. Cardiac complications, coronary disease, and acute myocardial ischemia are often present in cases of Fabry disease, frequently causing death in such patients. Characteristic central nervous system symptoms with skin-burning sensation and paresthesia were also present. Cerebrovascular complications were caused by changes on small blood vessels. Clinical signs of renal failure were nonspecific, and the diagnosis was based on extrarenal symptoms. Initial renal manifestations were insignificant as asymptomatic proteinuria and microhematuria, due to was referred to further examination. The level of alpha-galactosidase was significantly decreased. The severity and progression of this disease depends on the level of alpha-galactosidase enzyme in serum and its catabolic effect.

The major debilitating manifestations of Fabry disease result from the progressive accumulation of globotriaosylceramide in the vascular endothelium, leading to ischemia and infarction, especially in the kidney, heart, and brain. The ischemia and infarction of small vessels are primarily due to vascular occlusion; however, evidence for a prothrombotic state has recently been published. In addition, early and substantial deposition of globotriaosylceramide occurs in podocytes, leading to proteinuria and, with age, in cardiomyocytes, causing cardiac hypertrophy and conduction abnormalities.

Patients are generally divided into two major groups on the basis of the absence or presence of residual alpha-Gal A activity: classic disease and milder, later-onset, atypical variants. With the availability of enzyme replacement therapy, prompt diagnosis and treatment of Fabry disease have assumed new importance. There is no comparable alternative to enzyme replacement therapy for this devastating, progressive disease. Future research should address the development of protocols for early diagnosis as well as optimal enzyme protocols for reversal, maintenance, and prevention of the underlying pathology of Fabry disease, especially for children, patients with compromised renal function, and those with cardiac disease. Enrollment of all patients in a Fabry disease registry is encouraged to increase knowledge of the natural history and complications of the disease and the efficacy of treatment regimens. The Fabry Registry is a global observational research platform established to define outcome data on the natural and treated course of this rare disorder. Participating physicians submit structured longitudinal data to a centralized, confidential database. A recent report [60] describes the baseline demographic and clinical characteristics of the first 1765 patients (54% males (16% aged < 20 years) and 46% females (13% < 20 years)) enrolled in the Fabry Registry (Tables **16**, **17**, **18**). The median ages at symptom onset and diagnosis were 9 and 23 years (males) and 13 and 32 years (females), respectively, indicating diagnostic delays in both sexes. Frequent presenting symptoms in males included neurological pain (62%), skin

Table 13. Significant Differences in Inflammatory Gene Allele Distributions Between Patients with Fabry Disease Versus Controls

Polymorphic variant	Genotype	Patients	Controls	p value
TNF-α [-308]	GG	84%	63%	0.038
IL-1α [-889]	CC	94%	21%	<0.001

Table 14. Significant Differences Between Two Polymorphic Variants or with an MSSI Sub-Score

Polymorphic variant	Polymorphic variant/score	p value
IL-10 [819]	IL-10 [-592]	<0.0001
IL-1β [3954]	IL-10 [-592]	0.041
IL-1β [3954]	IL-1β [511]	0.001
IL-1α [-889]	IL-1β [3954]	0.002
IL-10 [819]	Neurological MSSI score	0.033

Table 15. Trends to Significant Difference Between Two Polymorphic Variants or with an MSSI Sub-Score

Polymorphic variant	Polymorphic variant/score	p value
IL-10 [819]	TNF-α [-308]	0.088
IL-10 [819]	IL-1β [3954]	0.069
IL-1α [-889]	TNF-α [-308]	0.095
TNF-α [-308]	Renal MSSI score	0.062
TNF-α [-308]	Neurological MSSI score	0.064

Table 16. Age at Onset and Types of Fabry Disease Symptoms

	Males (n=713)		Females(n=430)	
Organ system	**Median age at onset (years)**	**Percentage of males with symptom**	**Median age at onset (years)**	**Percentage of females with symptom**
Overall	9		13	
Neurological	9	67	10	47
Neurological: pain	9	62	10	47
Neurological: other	8	12	12	12
Skin	9	31	17	12
Ophthalmological	9	11	16	12
Gastroenterological	8	19	14	13
Respiratory	11	3	30	2
Renal	20	17	28	11
Cardiovascular	12	13	32	10
Cerebrovascular	10	5	26	4

Table 17. Age at First Clinical Event

		Fabry registry patients	
Clinical event		**Males (n=910)**	**Females (n=775)**
Renal event	% of patients	13	2
	Age(SD)	38.5(11)	37.3(10)
	MEDIAN(RANGE)	38(14-79)	38(17-59)
Cardiovascular event	% of patients	19	14
	Age(SD)	39.0(12)	47.6(13)
	Median(range)	41(5-76)	47(14-78)
Cerebrovascular event	% of patients	7	5
	Age(SD)	38.6(12)	43.2(14)
	Median(range)	38(18-80)	43(19-67)

Table 18. Age at diagnosis

	Males (n=882)	Females(n=718)
Mean (SD)age(years)	25.7(15)	32.2(18)
Median age(range)(years)	23(0-81)	32(0-80)
Distribution(%)		
0 to<10 years	16	9
≥10 to <20 years	25	16
≥20 to <30 years	19	17
≥30 to <40 years	18	17
≥40 to <50 years	12	17
≥50	7	16
Unknown	3	7

signs (31%), gastroenterological symptoms (19%), renal signs (unspecified) (17%), and ophthalmological signs (11%). First symptoms in females included neurological pain (41%), gastroenterological symptoms (13%), ophthalmological (12%), and skin signs (12%). For those patients reporting renal progression, the median age at occurrence was 38 years for both sexes, but onset of cerebrovascular and cardiovascular events was later in females (median 43 and 47 years, respectively) than in males (38 and 41 years, respectively).

These findings demonstrate that in spite of the considerable burden of disease in both sexes that begins to manifest in childhood or adolescence, the recognition of the underlying diagnosis is delayed by 14 years in males and 19 years in females and that AFD is often a multiorgan disease. This awareness could offer a possible way that can increase detection of common symptoms in all age groups, as well as insight into treated and untreated disease course, leading to improved recognition and earlier treatment, and possibly to improved outcomes for affected individuals.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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