Clinical Characterisation of Cardiac Involvement in Anderson-Fabry Disease.

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A thesis submitted to University College London for the degree of Doctor of Medicine (Research)

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Declaration

I, Jaymin Shantilal Shah confirm that the work presented in this thesis is my own. The data presented represents my own clinical assessment of the patients, data collection, management and analysis. The thesis presents my own original ideas and hypothesis. This work also reflects my efforts in coordinating the collaboration with multiple different centers to present a systematic clinical evaluation of Anderson – Fabry disease related cardiomyopathy. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Dr Jaymin S Shah

Abstract

Background

Anderson – Fabry Disease (AFD) is an X-linked lysosomal storage disorder that results in a deficiency in lysosomal α-galactosidase A. This leads to an accumulation of glycosphingolipids in multiple cell types resulting in the characteristic angiokeratomata, acroparesthesiae, hypohidrosis, and corneal opacities of classical AFD. Although patients with AFD have a reduced life span, the pathogenesis and impact of cardiac involvement on prognosis remain unclear. The aim of this thesis was to clinically characterise cardiac involvement in AFD using a systematic cardiac assessment of a consecutive cohort of patients with AFD.

Methods

A cardiac assessment protocol including history and examination, 12 lead ECG, 24 hour ambulatory ECG monitoring, echocardiography and cardiopulmonary exercise testing were performed in a cohort of 122 consecutive patients**.** Subgroups had coronary flow reserve and evaluation of serum levels of MMP-9, TIMP-1 and TIMP-2 to assess coronary microvascular function and extracellular matrix (ECM) turnover, respectively.

Results

The main results of the thesis are as follows: (1) Tachy and brady arrhythmias are common and may contribute to premature death; (2) Left ventricular systolic impairment is common and decline in systolic function may represent a measure of disease severity; **(**3) objective evidence for exercise limitation is common and is

related to overall disease severity as assessed with a validated disease severity score; (4) patients with AFD have markedly abnormal coronary microvascular function which does not improve with ERT; (5) patients with AFD have abnormal ECM turnover associated with overall disease severity.

Conclusion

Patients with AFD related cardiomyopathy have progressive disease characterised by heart failure and arrhythmia. It is likely that myocardial ischaemia and abnormal interstitial turnover are related in the pathogenesis of cardiac disease and complications. Patients with AFD need regular and continued cardiac follow up for early identification and treatment of cardiac complications.

Preface

Anderson – Fabry Disease (AFD) is an X-linked lysosomal storage disorder that results in a deficiency in the activity of lysosomal α-galactosidase A, leading to an accumulation of glycosphingolipids in multiple different cell types. This in turn causes the characteristic angiokeratomata, acroparesthesiae, hypohidrosis, and corneal opacities of classical AFD. AFD related heart disease is common, but its impact on prognosis remains unclear.

When I started my work in AFD, the evidence for cardiac involvement in AFD was in the form of case reports and case series. There had been few systematic studies to look at the prevalence of left ventricular failure, arrhythmia and exercise limitation in patients with AFD. Our collaboration with The Lysosmal Disorders Unit, The Charles Dent Metabolic unit and our own tertiary referral population of patients with unexplained left ventricular hypertrophy provided a unique opportunity to characterise cardiac involvement in AFD. This thesis is a systematic and comprehensive clinical cardiac evaluation of patients with AFD. The data presented are contemporary with my period as a research fellow. Since completion of my work numerous studies on AFD related heart disease have been published. I put my own work into the context of these data in the discussion.

Acknowledgements

The work in this thesis was undertaken during my period as a clinical research fellow at The Heart Hospital, University College London Hospital. The following have greatly assisted in its completion:

Professor Perry M Elliott: He introduced me to the field of hypertrophic cardiomyopathy. His support and supervision in the planning, development and writing of this thesis and manuscripts related to the research has been invaluable. His unstoppable drive, passion and vision have been an inspiration. He was not only a supervisor and mentor, but also a friend. I am honoured to have had the opportunity to have worked with him.

Professor William McKenna: for his supervision and constructive criticisms of the project.

The Research Team at The Heart Hospital: for their continued support throughout my time as a research fellow. They all have been invaluable sources of wisdom and have provided a steadying influence through all the phases of this project.

The staff of The Lysosomal Disease Unit, The Royal Free Hospital and The Charles Dent Metabolic Unit, The National Hospital for Neurology and Neurosurgery: For their continued support throughout my time as a research fellow and beyond.

Colleagues at City Hospital, Birmingham: For their expertise in the extra cellular matrix.

Colleagues at MRC Clinical Sciences Centre, Imperial College, London: For their expertise in coronary flow reserve

TKT-5S (Now Shire Human Genetic Therapies) and Sheila Bone: For funding my research and providing me with the opportunities that have resulted in this work.

My wife and family: For their support and forgiveness for 'bringing work home'.

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List of abbreviations

AFD	Anderson Fabry Disease
ECG	Electrocardiogram
MMP	Matrix metalloproteinase
TIMP	Tissue inhibitors of metalloproteinase
ECM	Extracellular matrix turnover
LSD	Lysosomal storage disorders
Gb3	Globotriaosylceramide
A-Gal	α – galactosidase A
FOS	Fabry outcome survey
AF	Atrial fibrillation
PAF	Paroxysmal atrial fibrillation
NSVT	Non-sustained ventricular tachycardia
NYHA	New York Heart Association
ERT	Enzyme replacement therapy
RE	Romhilt-Estes score
LVed	End diastolic left ventricular internal cavity diameter
LVes	End systolic left ventricular internal cavity diameter
IVS	Interventricular septum
PW	Posterior wall
RWT	Relative wall thickness
FS	Fractional shortening
LV	Left ventricular
LVH	Left ventricular hypertrophy

HCM	Hypertrophic cardiomyopathy
CPET	Cardiopulmonary exercise testing
pVO_2	Oxygen consumption at peak exercise
$\%VO_2max$	Per cent of predicted oxygen consumption at peak exercise
MSSI	Mainz severity score index
VCO_2	Carbon dioxide output
RQ	Respiratory quotient
AT	Anaerobic threshold
O_2P	Oxygen pulse
SV	Stroke volume
A-V	systemic arteriovenous oxygen difference
CFR	Coronary flow reserve
MBF	Myocardial blood flow
PET	Positron emission tomography
ARSAC	Administration of radioactive substances advisory committee
RPP	Rate pressure product

Chapter 1

Introduction

Introduction

Lysosomal storage disorders (LSD) are a distinct group of diseases that are the result of genetic defects in the genes encoding lysosomal enzymes or structural proteins[1]. Absent or reduced enzyme levels result in the accumulation of lysosomal storage products that would normally be degraded by the affected enzyme. The diseases are categorised according to the type of storage product that accumulates (e.g. mucopolysaccharidoses and glycoproteinoses[2]).

LSD are characterised by a progressive disease course and premature death. They are individually rare with population incidences ranging from 1 in 57 000 live births for Gaucher's disease and 1 in 4.2 million live births for Sialidosis[3]; however as a group their incidence has been estimated as 1 in 7000-8000 live births[3, 4].

The lysosome

In the late 1950s and 60s, Christian De Duve, using cell fractionation techniques, cytological studies and biochemical analysis, characterised the lysosome as the intracellular organelle responsible for the degradation and recycling of macromolecules[5-7]. This scientific breakthrough led to the physiological understanding of LSDs, with Pompe's disease being the first to be identified in 1963 by Hers and colleagues[8, 9]. Much subsequent research has focused on diagnosis (both prenatal and postnatal), carrier identification and the development of specific therapies.

Anderson – Fabry disease

AFD is an X-linked lysosomal storage disorder resulting in systemic accumulation of lysosomal globotriaosylceramide (Gb3) due to a deficiency in lysosomal α-galactosidase A (A-Gal) activity[1]. AFD is unlike most other X-linked conditions as it can affect both genders although the course of the disease in females may be delayed in presentation[1]. The disease is characterised by progressive clinical manifestation of multiple organ system involvement, with symptoms usually presenting in childhood, and premature death from stroke, renal or cardiac involvement[10] (Figure 1.1).

The history of Anderson-Fabry disease

The first descriptions of AFD were made simultaneously by two independently working physicians in 1898. William Anderson and Johannes Fabry described 'angiokeratoma corporis diffusum', the characteristic cutaneous manifestation of AFD (Figure 1.2) [11, 12].

William Anderson, who trained at St Thomas's Hospital, saw his first patient with the rash, without commenting on the underlying cause. Johannes Fabry was a dermatologist at the University of Bonn. He described a 13 year old boy who had previously developed an eruption starting at the back of his left knee and spreading to his thigh. He thought that the disease may be a form of naevus or represent a developmental defect. Both these physicians saw their patients in 1897 and in recognition of this the disease is sometimes referred to as Anderson – Fabry disease.

Figure 1.1: Disease course in classical AFD

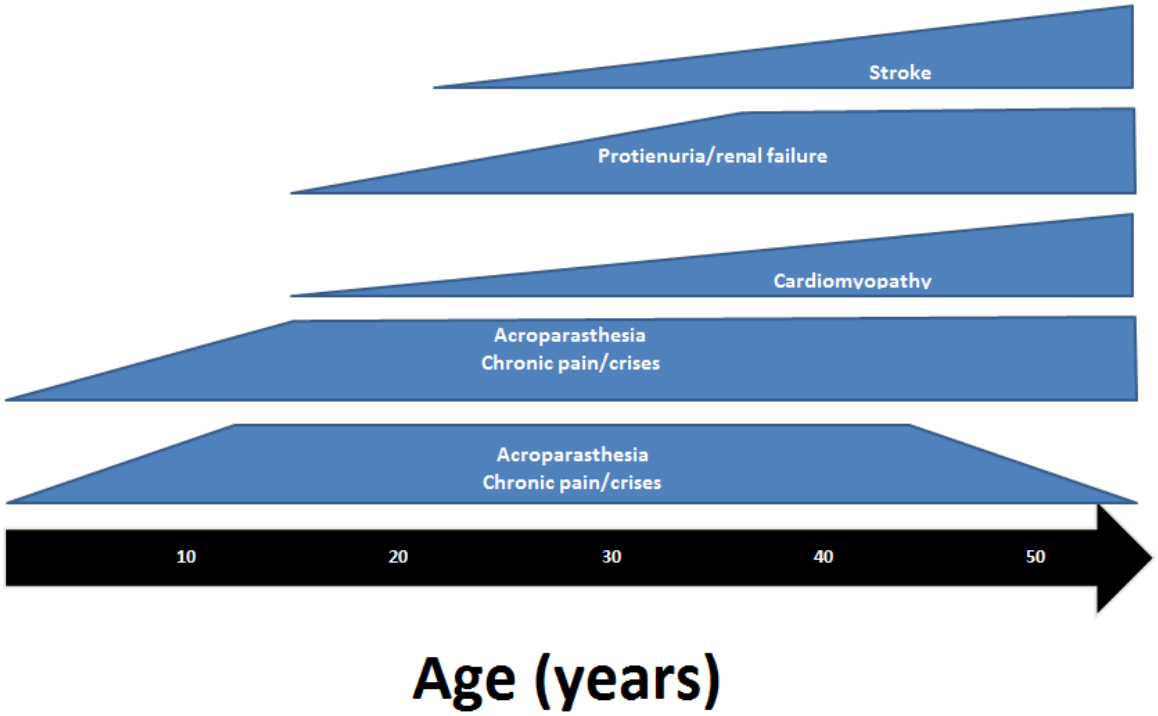


Figure 1.1 legend: adapted from Linhart *et al*[13]

Figure 1.2: Johannes Fabry and William Anderson

Figure 1.2 legend: Johannes Fabry and William Anderson respectively

Between 1909 and 1939 many physicians described the multi-organ involvement in AFD with a suspicion that there was a common cause behind all the symptoms[14-16]. In 1947, an autopsy demonstrated changes in the entire vascular system with accumulation of a peptide like substance[17]. These findings were confirmed in another autopsy in 1950 where birefringent Maltese-cross-like structures under polarized light were noted as a typical pathological feature and characterised as phosphoglycolipid[18]. It was until the late 50s that it was felt that AFD only affected males but in 1959 Colley reported possible involvement in females[19]. This was contrary to prevalent literature at the time[20]. In 1964 de Groot published on a single family of 45 individuals spanning 4 generations (including females with minimal symptoms) and characterised AFD as a dominant hereditary disturbance of lipid metabolism. He commented on the discrepancy between severity of symptoms in females as opposed to males was incongruous with it being a dominant hereditary disturbance[21].

With the use of an electron microscope, Hashimoto et al identified the lysosome in a patient with AFD and commented on the lysosomes being 'extremely overcrowded'[22]. In this study he concluded that the cause of AFD was a genetic disturbance in a lysosomal enzyme. In the same year it was realised that AFD was a sex linked genetic enzyme deficiency. It was noted that there was occasional penetrance in heterozygous females and constant penetrance in hemizygous males[23]. Additionally in the same year the genetic deficit was isolated to the long arm of the X chromosome (locus Xg)[24].

In 1967 the enzyme deficiency of ceramidetrihexosidase and subsequent substrate accumulation was identified as the underlying cause of AFD[25]. In 1972, it was reported

that AFD was corrected with kidney transplantation[26], subsequently the treatment of a patient with purified ceramidetrihexosidase was described in 1973[27].

The epidemiology of AFD

Reported prevalence of AFD ranges from 1 in 476,000 to 1 in 117,000 in the general population[3, 4], based on the total number of cases identified in a particular period divided by the total number of births in that same period. These figures have important limitations due to the nature of the studies, the failure to detect asymptomatic carriers (particularly women) and misdiagnosed cases resulting in significantly under estimated figures. Table 1.1 demonstrates that systematic new born screening reveals a higher incidence than thought previously. The consistently high incidence of AFD in these systematic new born screening studies suggests that AFD is pan ethnic[28-32] and suggest that the cardiac predominant genotypes may be more common than previously estimated[29, 33].

The tests used in these studies, for new born screening (flourometry, flow-injection tandem mass spectrometry or digital micro fluidics) have a positive predictive value ranging from 3.8 to 54% with appreciable false positive rates[34]. There is a lack of prospective new born screening and treatment studies in AFD and uncertainty regarding effectiveness of and immunologic response to treatment make this kind of screening controversial.

Table 1.1: Studies of prevalence of AFD

Type of study	Study period	Number per 100,000	Country
Birth prevalence	1980-1996	0.85	Austrailia[3]
Birth prevalence	1970-1996	0.21	Netherlands[4]
Prevalence of obligate carriers (females)	1980-1995	0.29	UK[35]
Prevalence	1980-1995	0.27	UK[10]
Birth prevalence	1997-2002	0.015	Turkey[30]
Birth prevalence	1982-2001	0.12	Portugal[31]
Neonatal screening	2004-2006	30	Italy[32]
Neonatal screening	2006-2008	80	Taiwan[33]
Neonatal screening	2007-2010	14	Japan[28]
Neonatal screening	2013 (6 months)	34	Missouri[29]

Table 1.1 legend: Table adapted from Germain[36]

Molecular genetics of AFD

AFD results from mutations in the α–galactosidase gene (GLA), located on the long arm of the X-chromosome (Xq22.1) which encodes a 101 kDa homodimeric glycoprotein that exists in a number of natural forms resulting from variations in sialic acid residues on carbohydrate chains[1]. Over 600 mutations have been described in all seven gene exons, the majority (57%) being mis-sense point mutations with some premature stop codons, splice site mutations and insertion/deletions[1, 37-42]. Reduced enzyme activity occurs by several mechanisms including abnormal/unstable protein folding, perturbation to active binding site, and defects in enzyme tracking to the lysosome[1, 40]. Despite this condition being described as an X-linked recessive disease, female carriers also develop symptoms and signs of the disease. This is thought to be related to the mechanism of non-random X chromosome inactivation (Lyonisation)[43]

Clinical presentation

Patients with AFD can present with non-specific symptoms, resulting in a delay in diagnosis. Data from the Fabry Outcome Survey (FOS), an International prospective registry study, suggest that there is a delay of 13.7 years for males and 16.3 years for females from the onset of symptoms to diagnosis[44]. Overall, 25% of patients in FOS have reported a previous misdiagnosis[44].

In the FOS study the most frequently reported signs and symptoms were neurological[44]. Both male and female patients had a high prevalence of neuropathic pain (76% and 64%, respectively) occurring with earlier age of onset in males than in females. Angiokeratomata occurred in 78% of males and 50% of females.

Table 1.2 Signs and symptoms of AFD

Organ system	Sign/symptom
Nervous system	Acroparesthesiae Tinnitus/hearing loss Heat intolerance Stroke
Gastrointestinal tract	Nausea, vomiting, bloating, diarrhoea (irritable bowel syndrome like symptoms) Difficulty gaining weight
Skin	Angiokeratomata Hypo/anhidrosis
Eyes	Corneal and lenticular opacities (‘cornea verticillata’)
Kidney	Microalbuminuria/proteinuria Hyperfiltration Renal failure requiring dialysis
Heart	Arrhythmia Left ventricular hypertrophy Heart failure Angina, breathlessness, palpitation, syncope, sudden death

Fifty percent of patients with AFD had signs and symptoms of renal disease, the commonest being proteinuria observed in 44% of males and 33% of females. End-stage renal failure was present in 17% of males and 1% of females. Women (27%) had a higher prevalence of cerebrovascular events than men (17%), with the events occurring at a younger age in males than in females. Auditory symptoms, such as tinnitus and hearing loss, were present in more than 50% of patients and ocular signs in just less than 60% at presentation. Gastrointestinal symptoms, including abdominal pain and diarrhoea were present in approximately 50% of patients with AFD. Fatigue was reported as a major symptom in approximately 20% of patients[44]. Table 1.2 describes the signs and symptoms of AFD stratified by organ system.

Neurology

Early neural damage affects the small nerve fibres of the peripheral somatic and autonomic nerve systems resulting in the characteristic acroparasthesiae, hypohidrosis and gastrointestinal symptoms[45, 46]. Patients describe two types of pain:

(a) 'Fabry crisis' – these are episodic crises of agonizing burning pains in the extremities radiating inward to the limbs and other parts of the body. These crises may be precipitated by fever, fatigue, exercise and rapid changes in temperature[47]. Crises are often accompanied with a raised erythrocyte sedimentation rate, resulting in misdiagnosis such as rheumatoid arthritis and Raynaud's phenomenon; (b) Chronic pain characterised by chronic burning and tingling paraesthesia[46]. Both types of pain often wane in adulthood and so it is important to question patients about childhood symptoms.

Cerebrovascular involvement in AFD can lead to a variety of symptoms and signs including headache, vertigo/dizziness, transient ischaemic attacks, ischaemic strokes and rarely vascular dementia[48, 49]. The potential pathophysiology behind cerebrovascular disease in AFD includes multifocal involvement of small vessels, increased thrombotic potential (measurable activation of the endothelium and leukocytes), dilative arteriopathy in the vertibrobasilar circulation and changes in regional cerebral hyperperfusion[50-52]. In one large study of patients with AFD, the prevalence of cryptogenic stroke was 6.9% in males and 4.3% in females with the median age at presentation of 39 in males and 46 in females[53].

Auditory and vestibular involvement results in a range of symptoms from hearing loss (progressive or sudden), tinnitus and vertigo[44]. The mechanism of these symptoms is likely to be related to both neuropathy and vasculopathy.

Gastrointestinal

The gastrointestinal symptoms are under-appreciated, they include post prandial abdominal pain, diarrhoea, vomiting and failure to gain weight[45]. These symptoms are caused by deposition of Gb3 in the autonomic ganglia of the bowel and mesenteric blood vessels[54].

Skin

The most visible clinical feature of AFD is angiokeratomata consisting of clusters of small reddish purple, and raised skin lesions (Figure 1.3). These are typically found on

the buttocks, groin, umbilicus and upper thighs and sometimes on mucous membranes[10, 55, 56]. Histologically, the skin lesions are small superficial angiomas caused by cumulative damage of the vascular endothelial cells of the skin with vessel dilatation in the dermis that increase in number and size with age and can occur singly or in groups[55, 56]. Anhidrosis and hypohidrosis are attributable to selective peripheral nerve damage or to intracytoplasmic lipid deposits in the sweat glands[57]. This results in exercise and heat intolerance[46].

Eye changes

Patients frequently have corneal changes ('*cornea verticillata*'), these are readily detectable on slit lamp examination, but do not have any impact on visual acuity (Figure 1.4)[58]. There are also descriptions of increased retinal vascular tortuosity[58].

Renal involvement

Renal involvement is a major cause of morbidity and mortality in AFD[44]. Like several aspects of AFD, renal involvement is progressive and increases in severity with age (Figure 1.1)[13]. Renal impairment is thought to be a direct consequence of GB_3 deposition in glomerular endothelial, mesangial, interstitial cells and podocytes[59]. Initially patients develop microalbuminuria and proteinuria in the 2nd and 3rd decades of life[60].

Figure 1.3: Angiokeratoma

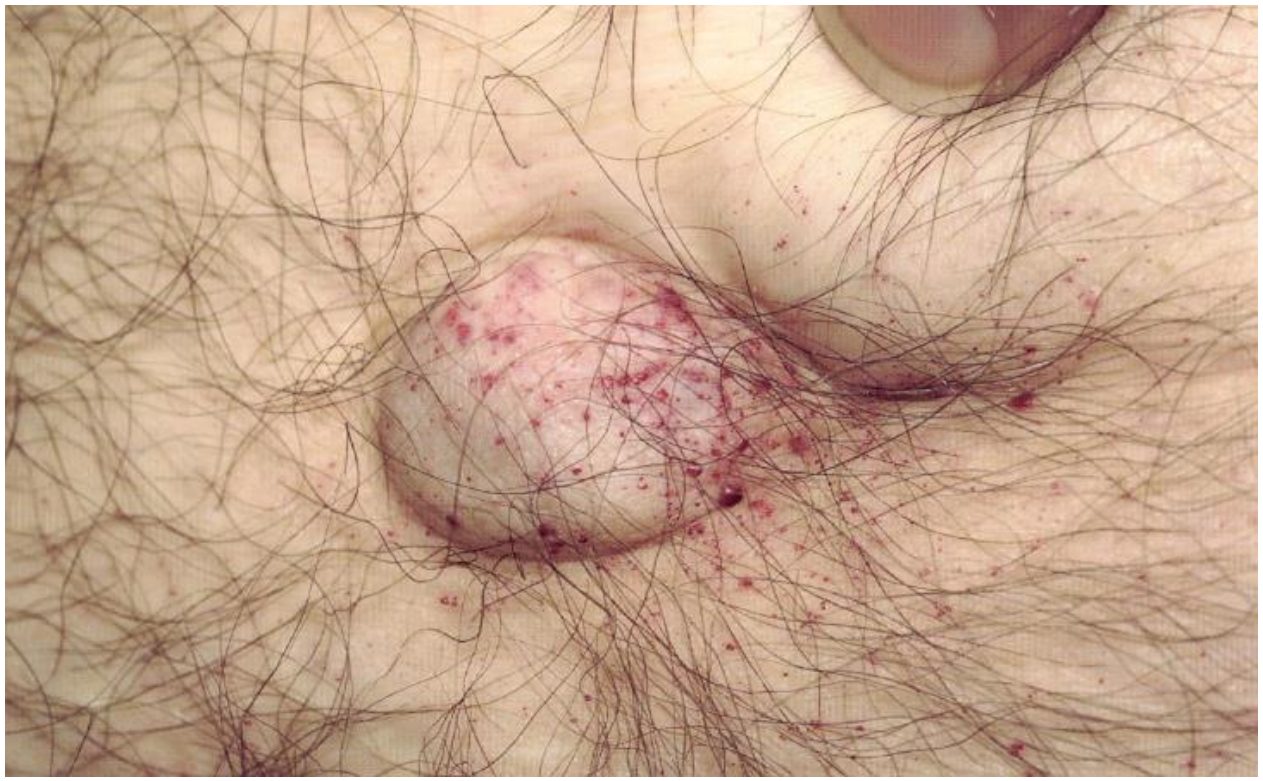
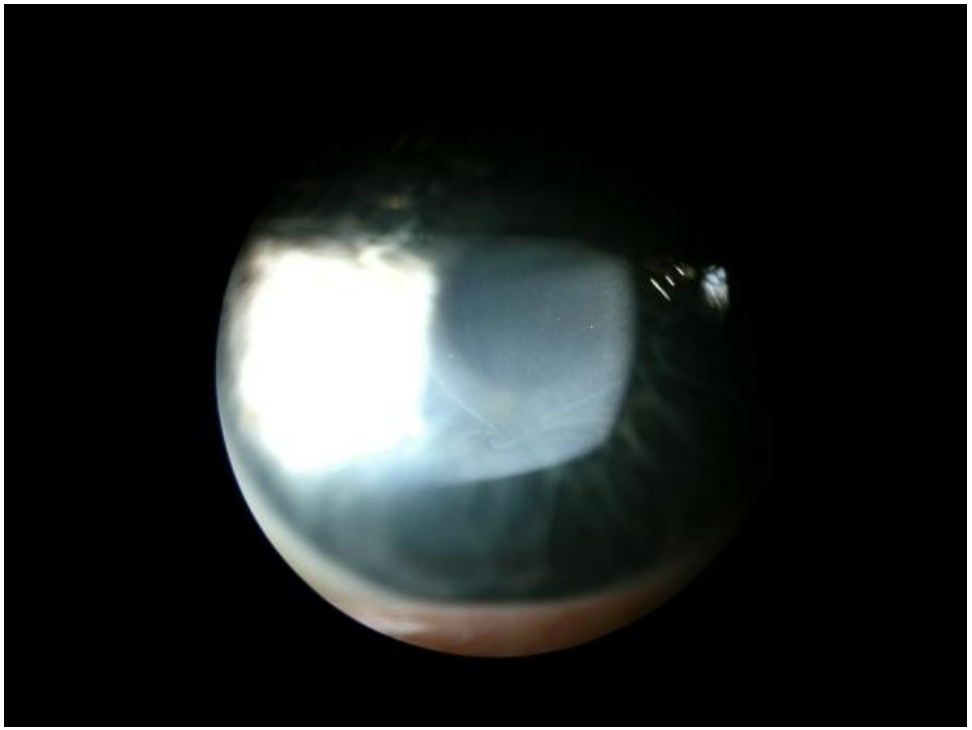


Figure 1.4: Cornea Verticillata



Progressive disease results in worsening proteinuria and a reduction in glomerular filtration, generally in the 3rd to 5th decade of life[60]. At this stage fibrosis, sclerosis and tubular atrophy predominate on histology with end stage renal disease occurring in 4th-5th decade of life[59, 60].

Cardiac disease in AFD

Cardiac involvement in AFD begins early (Figure 1.1), with the average age for clinically overt symptoms being 32 years in the male and 40 years in the female [44]. Cardiac disease in AFD, as with other organ systems is associated with progressive Gb3 accumulation in all cellular components of the heart. Histological studies have identified GB3 in cardiomyocytes, conduction system cells, valvular fibroblasts, endothelial cells and vascular smooth muscle cells [61, 62].

Cardiomyocytes are vacuolated and hypertrophied, but unlike familial hypertrophic cardiomyopathy, myofibrillar disarray is not prominent [62]. Lysosomal inclusions are present within myofibrils and vascular structures; fibrosis is evident within the mid-myocardial layers and the posterolateral segments of the left ventricle (Figures 1.5 and 1.6) [63]. While Gb3 accumulation is the most prominent feature histologically, it represents only 1–2% of the total cardiac mass[64]. Therefore, it is likely that disease in the heart results from activation of other signalling pathways that lead to hypertrophy, apoptosis, necrosis and fibrosis. These progressive changes correlate with observations of relatively mild diastolic dysfunction in early stages of the disease progressing to systolic and severe diastolic ventricular impairment in advanced disease[64].

Figure 1.5: AFD cardiomyopathy histology

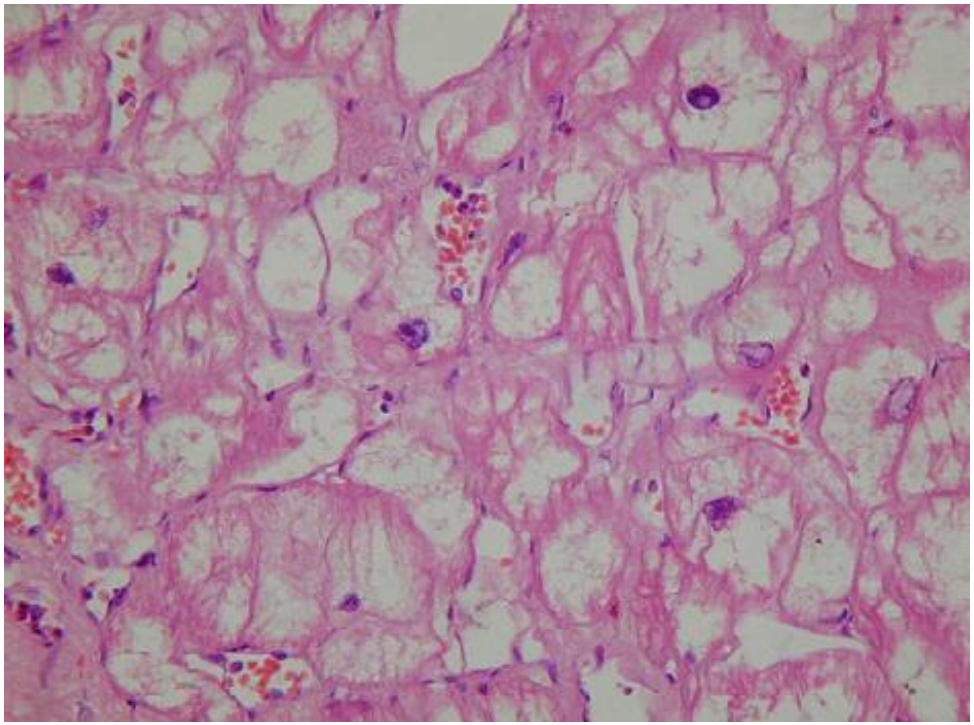


Figure 1.5 Legend: Haematoxylin and eosin stain (x100) demonstrating myocyte hypertrophy and vacuolization from GB3 accumulation within the cell.

Figure 1.6: Fibrosis in Fabry disease cardiomyopathy

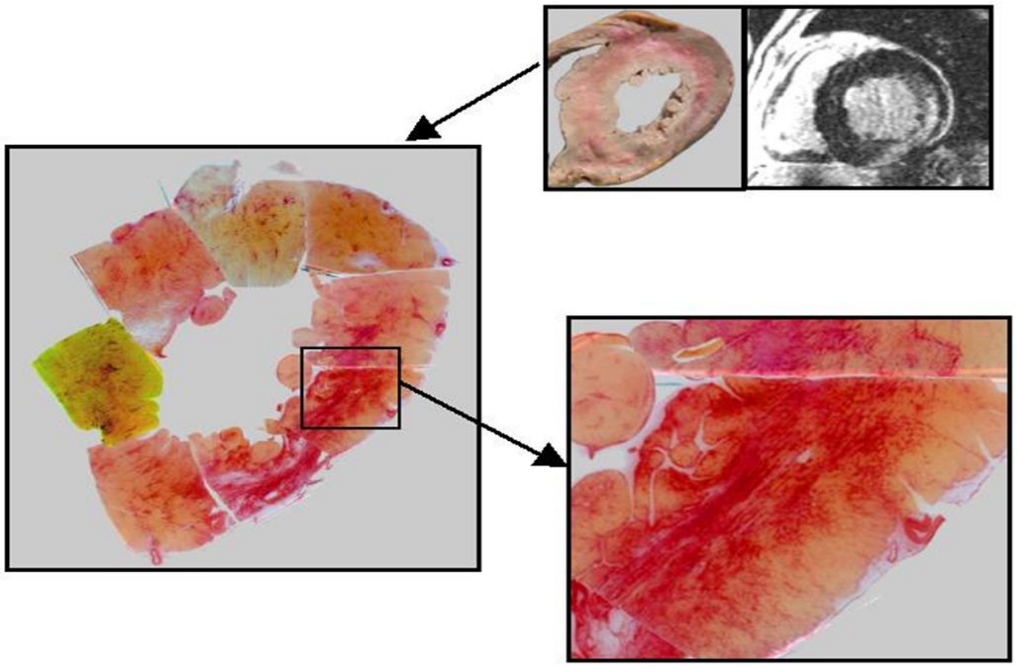


Figure 1.6 legend: Localised myocardial scar seen on cardiac MRI scan correlated to myocardial fibrosis on Picro-sirius red staining at post mortem examination of the same heart, in the absence of significant coronary artery disease

The 'cardiac variant'

Patients with residual enzyme activity (1-5%) can have a delayed presentation in middle age with predominantly cardiovascular symptoms, LVH, cardiac rhythm abnormalities and valve disease[65-67]. However, while there is an absence of other 'classical features', rigorous clinical characterisation usually reveals organ involvement elsewhere. Hence AFD should be considered in all patients with otherwise unexplained left ventricular hypertrophy from the middle decade of life.

Heart disease in females

Clinical manifestations have generally been thought to be rare or mild in female carriers. However, data from the Fabry Outcome Survey (FOS) and other sources show that most patients have signs or symptoms of disease with a similar prevalence of fatigue, neurological, gastrointestinal signs and symptoms in men and women[10, 35, 44]. Despite a lower prevalence of left ventricular hypertrophy, females have a similar prevalence of cardiac symptoms such as angina, dyspnoea and palpitations[44]. The prevalence of AFD related symptoms and signs increase with age in both males and females and it is suggested that females with AFD have a 15 year reduction in their life span when compared to the general population[35]. A study by Kampmann *et al* which examined 55 females with AFD confirmed on mutational analysis showed that the severity of the cardiac involvement in this group of patients may be as great as in that of males[68]. A twin study has suggested the mechanism of non-random X inactivation (Lyonisation) may result in disease expression in females[43]. This hypothesis is further strengthened by a recent study that demonstrates a higher Mainz Severity Score (a

validated severity score for disease severity) in females with non-random X inactivation demonstrated in peripheral blood compared to those without[69].

Enzyme replacement therapy

Exogenous proteins result in the possibility of sensitising the patient with a resulting reduced efficacy or allergy to the protein, hence initial efforts at enzyme replacement therapy (ERT) focused on a human source of A-Gal. Enzyme extracted from human placental tissue was infused into 2 patients with AFD it resulted in a rapid reduction in circulating Gb3, returning to pre-infusion levels in 48 hours[27]. Additional studies were carried out with A-Gal extracted from spleen and plasma with promising results[70]; however it became apparent that these sources would only provide limited quantities of the enzyme. This resulted in 2 biotechnology corporations synthesising recombinant A-Gal. Transkaryotic Therapies, Inc., Cambridge MA, subsequently known as Shire Human Genetic Therapies, Inc. A-Gal was initially produced in a cultured human skin fibroblast cell line using a proprietary gene-activation technique. Known as *Agalsidase–alfa* (Replagal™) it is administered in a dose of 0.2 mg/kg of body weight every second week in intravenous (iv) infusions of 40 min. Initial studies with this infusion resulted in > 30% reduction in hepatic and urinary Gb3[71]. A randomised controlled trial by Schiffmann *et al* demonstrated a significant improvement in neuropathic pain, renal function, cardiac conduction and weight gain[72].

The Genzyme Corporation, Cambridge, MA, used transduced Chinese hamster ovary cells to produce recombinant a-galactosidase A, *Agalsidase–beta* (Fabrazyme^{TM}), also administered on a biweekly basis, but in a dose of 1 mg/kg in a 4–6 h iv infusion. The initial trial with agalsidase beta revealed clearance of Gb3 from the kidney, skin and heart and reduction of plasma and urinary Gb3 levels[73]. This study demonstrated that 88% of patients developed antibodies to the enzyme, but it was felt that this did not affect the efficacy end points[73].

Several studies using both enzyme products have demonstrated an improvement in renal, neurological and quality of life improvements[72, 73]. There has been a phase three trial that has demonstrated a reduction in QRS duration on ECG, cardiac structural and functional improvement and clearance of GB3 from the vascular endothelium[72, 74, 75]. However, post mortem studies have demonstrated continued GB3 storage within myocytes despite several years of enzyme replacement therapy[76]. The true prognostic effect of enzyme replacement therapy remains to be determined.

Aims of the thesis

Exercise limitation

Male and female patients with AFD have an equal prevalence of cardiac symptoms, including effort intolerance, despite differences in the severity of cardiac disease at presentation[68, 77, 78]. The mechanism and clinical significance of exercise limitation in patients with AFD have not been studied in detail. I will determine the exercise capacity

in a large referral population of patients with AFD and its relation to markers of disease severity.

Left ventricular function

I have described above that there are data to support progressive left ventricular hypertrophy. Weidemann *et al*. demonstrate that enzyme replacement therapy improves cardiac contractile function as measured by strain rate on echocardiography, however there are no data to document the natural history of left ventricular systolic function in AFD[74]. We have long term follow up data on a large cohort of AFD patients allowing the description of the above and its relationship to symptoms.

Arrhythmia

The majority of adult patients with AFD will have abnormal resting ECGs, the most common abnormalities being voltage criteria for left ventricular hypertrophy and repolarisation abnormalities. Short PR intervals, usually in the absence of an accessory pathway, AV conduction disease, sinus node disease and bundle branch block have also been described[68, 77, 78]. Nevertheless, the importance of cardiac arrhythmia in the natural history of AFD is unknown. I will describe the prevalence of arrhythmia in a prospectively studied cohort of patients with AFD, and determine its impact on clinical outcomes.

Pathogenesis

Myocardial fibrosis has been described as a prominent feature of AFD cardiomyopathy, however there are little data regarding the pathogenesis of this and its relationship to symptoms and overall disease severity[63]. I will investigate myocardial ischaemia and collagen turn over and their relation to symptoms and disease severity.

Summary of my role in the research project

Together with Professor Perry Elliott, I developed the idea for the research project presented in this thesis and obtained funding for it. I was responsible for recruiting patients into the project, and carried out the clinical and echocardiographic evaluation of patients recruited between 2002 and 2005. I also retrospectively entered clinical and echocardiographic data onto the database for all patients recruited into the study, including all patients evaluated before 2002, by manually searching through patients' medical records, echocardiograms and other investigations. In addition, I modified the database so that data relevant to the AFD population could be entered and analysed. I carried out all the analyses presented in this thesis.

Chapter 2

Characterisation of cardiac arrhythmia

Abstract

Background

Over 60% of patients with AFD have symptoms and signs of cardiac involvement. The prevalence and clinical significance of arrhythmia in AFD are unknown.

Methods and results

The patient cohort comprised seventy-eight patients (43 male, 43.5 ± 15.0 years, 13.0-83.0 years) with AFD, studied for a mean of 1.9 years (0.25-10 years). All patients underwent clinical evaluation, 12-lead ECG and echocardiography. Sixty patients (76.9%) underwent 24 hour ambulatory ECG (Holter) monitoring. Atrial fibrillation (AF) was seen at baseline in 3/78 (3.9%) patients. On Holter monitoring 8 (13.3%) had paroxysmal AF (PAF) and 5 (8.3%) had non-sustained ventricular tachycardia (NSVT). Patients with NSVT were all male with a maximal left ventricular wall thickness > 20mm. Univariate analysis identified age ($p<0.001$), left atrial diameter ($p = 0.001$), maximal left ventricular wall thickness ($p = 0.003$), left ventricular mass index ($p = 0.009$) and angina ($p = 0.02$) as predictors of AF/PAF. Stepwise logistic regression identified age as the only independent predictor of AF/PAF (OR = 1.2, 95% CI = 1.1 to 1.3, $p = 0.001$). Four patients had pacemakers implanted for bradyarrhythmia and 1 had a bi-ventricular pacemaker and internal cardioverter defibrillator implanted for heart failure and symptomatic NSVT.

Conclusion

Brady- and tachyarrhythmias are common in older patients with AFD. Patients with AFD should undergo regular cardiological review, as the development of arrhythmia in these patients may have an impact on the natural history of this disease.

Aims

The aims of this study were to determine the prevalence of cardiac arrhythmia in a large consecutively referred population of patients with AFD, and to assess their clinical significance.

Methods

Between January 1993 and December 2003, 78 patients (43 (55.1%) male; 35 (44.9%) female; mean age ($\pm$ SD) = 43.5 $\pm$ 15.0 years; range = 13-83) with AFD were assessed at The Heart Hospital, University College London, UK. Patients were diagnosed with AFD on the basis of low plasma α-Gal level and / or on mutational analysis. Seven patients had been identified during the screening of patients with unexplained left ventricular hypertrophy [79]. The remaining patients were direct referrals for cardiac evaluation from The Charles Dent Metabolic Unit, The National Hospital for Neurology and Neurosurgery and The Lysosomal Storage Disorders Unit, The Royal Free Hospital, London, UK. Forty-one patients (6 female, 35 male) were on enzyme replacement therapy (ERT) at the base line visit.

Clinical Assessment

All patients underwent clinical history and examination. Cardiac symptoms were defined as angina (central chest pain occurring during exertion, lasting for less than 15 minutes), dyspnoea (graded using New York Heart Association criteria; NYHA), syncope and palpitation.

ECG Assessment

All patients had a standard supine electrocardiogram (ECG) performed using a Hewlett-Packard Page Writer, Andover, MA, USA. ECGs were recorded at 25 mm/s with an amplitude of 1 mV/10mm and 50 Hz filtering. The following features were recorded: Rhythm, PR interval and QRS duration. LVH was assessed using the Romhilt-Estes (RE) score [80]. A normal P-R interval was defined as > 120ms and ≤ 200ms. A prolonged QRS duration was defined as > 120ms.

Twenty-four hour ambulatory ECG (Holter) was performed in 60/78 (76.9%) patients using Reynolds Medical tape Holter monitors, Hertford, UK. Non-sustained ventricular tachycardia (NSVT) was defined as three or more consecutive ventricular ectopics at a rate > 120 beats per minute lasting less than 30 seconds. Atrial fibrillation (AF) was defined on the basis of the baseline ECG showing the replacement of consistent P waves by rapid oscillations or fibrillatory waves that vary in size, shape, and timing [81]. Paroxysmal atrial fibrillation (PAF) was defined as episodes of AF lasting less than seven days, diagnosed from documented arrhythmia on 24 hour ambulatory ECG [82]. Persistent AF was defined as AF lasting more than seven days, but potentially amenable to cardioversion[82]. Permanent AF was defined as AF in which chemical/electrical cardioversion failed or was not attempted[82].

Echocardiography

M-mode, 2D, and Doppler echocardiography were performed using a GE System V echocardiograph. The following left ventricular parameters were measured from M-

mode tracings according to recommendations of the American Society of Echocardiography: end-diastolic left ventricular internal cavity diameter (LVed), interventricular septum thickness (IVS), posterior wall thickness (PW) and end systolic internal cavity diameter (LVes)[83]. The severity and distribution of left ventricular hypertrophy were assessed in the parasternal short-axis plane at the mitral valve and papillary muscle level. Maximum left ventricular wall thickness was defined as the greatest thickness in any single segment measured in diastole. Relative wall thickness (RWT) was calculated as ((IVS + PW)/LVed) assessed at the mitral valve level. Left Ventricular remodeling or left ventricular hypertrophy was defined as a RWT > 0.45[83]. Left ventricular mass was calculated by the Devereux modified cube formula [83] and indexed by body surface area. Normal left ventricular mass index (LVMI) was defined as <134 gm/m^2 for men and <110 gm/m^2 for women [84]. Left ventricular geometry was classified as normal (normal LVMI and normal RWT), concentric remodelling (normal LVMI and increased RWT), eccentric left ventricular hypertrophy (increased LVMI and normal RWT), and concentric left ventricular hypertrophy (increased LVMI and increased RWT). Fractional shortening (FS) ((LVed-LVes/LVed)*100) was used to measure systolic performance[85].

Follow Up

Follow-up started with the date of the baseline cardiovascular assessment. Data were collected at routine clinic visits, and where necessary by direct communication with patients, their attending physicians and patients' general practitioners; all data were verified by the author.

Statistical Analysis

Statistical analysis was performed using SPSS v19.0. The χ^2 test was used to compare non-continuous variables. Continuous variables were tested for normality, non-normally distributed variables were tested using the Wilcoxon Rank sum and normally distributed variables compared using the 2-tailed independent sample Student's *t*-test. All values are expressed as means $\pm$ standard deviation. Statistical significance was defined as $p < 0.05$.

Results

Clinical Evaluation

Table 2.1 describes the prevalence of cardiac and AFD symptoms separated by gender. Table 2.2 displays the important findings in the baseline electrocardiographic and echocardiographic findings for this cohort. There were no differences between males and females in the prevalence of cardiac symptoms. Men were more likely to suffer from AFD symptoms and signs compared to women.

ECG Changes at Baseline

There were no significant differences between men and women in the baseline heart rate, QRS axis or P-R interval. The mean RE score was greater (mean difference = 2.8, 95% CI = 1.7 to 4.0, p = <0.001) and the mean QRS duration was greater (p < 0.001) in men compared to women (see Table 2.2). Two males had evidence of pre-excitation on their baseline ECG. One of these patients had an intracardiac Electrophysiological study. No accessory pathway was identified; however there was accelerated AV

conduction with a fixed HV time of less than 10ms and normal decremental AV and VA conduction. The second patient went into complete heart block and there was evidence of a presumed accessory pathway on his Cardiomemo ECG as demonstrated by a pre-excited QRS complex followed by a normally conducted QRS complex (Figure 2.1). Three of the 78 patients had AF (3.9%; 2 persistent and 1 permanent). There was no difference in the prevalence of AF in patients on ERT compared to those not on ERT.

Holter assessment at baseline

Sixty patients underwent Holter assessment at the baseline visit. Eight of the 60 patients (13.3%) had PAF (lasting less than 24 hours). Five of the 60 patients (8.3%) had NSVT. There was no difference in the prevalence of AF/PAF between males and females. There seemed to be no difference in the prevalence of PAF/NSVT on Holter between patients on ERT and those not on ERT. There was a significant increase in the prevalence of arrhythmia with age (Figure 2.2a and b).

Echocardiographic Parameters at Baseline

Compared to females, males had greater maximal left ventricular wall thickness (mean difference = 3.6mm, 95% CI = 1.6, 5.6mm, p = <0.001), larger LVed (mean difference =3.9 mm, 95% CI = 1.6, 6.2mm, p = 0.001) and higher LVMI (p < 0.001, see Table 2.2). There was a trend to a larger LA size and LVes in men compared to women (p = 0.07 and p = 0.08 respectively; Table 2.2).

Table 2.1: Symptoms at Baseline Evaluation in Males and Females

	All (n=78)	Female (n=35)	Male (n=43)	p-value
Chest Pain	**15 (19%)**	**8 (23%)**	**7 (16%)**	**0.57**
Dyspnoea*	**22 (28%)**	**7 (20%)**	**15 (36%)**	**0.21**
Palpitations	**22 (28%)**	**10 (29%)**	**12 (28%)**	**1.0**
Syncope	**6 (8%)**	**2 (6%)**	**4 (9%)**	**0.7**
Acroparaesthesiae	**45 (58%)**	**14 (40%)**	**31 (72%)**	**0.005**
Hypohydrosis	**24 (31%)**	**5 (14%)**	**19 (44%)**	**0.006**
Angiokeratoma	**27 (35%)**	**4 (11%)**	**23 (54%)**	**<0.001**
Abdominal Pain	**22 (28%)**	**6 (17%)**	**16 (37%)**	**0.048**
Tinnitus	**15 (19%)**	**1 (3%)**	**14 (33%)**	**0.001**

Table 2.1 Legend: * = New York Heart Association class II and above.

Figure 2.1: Complete heart block

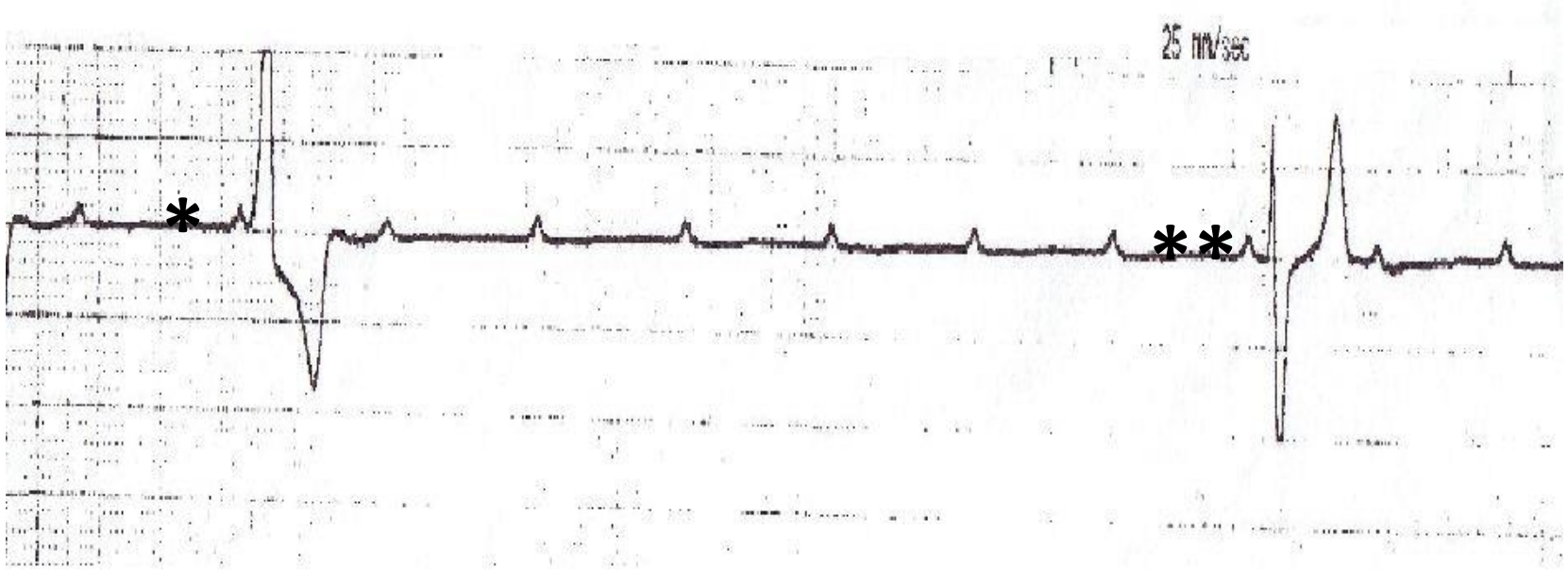


Figure 2.1 Legends: Cardio-memo strip showing complete heart block in a 55-year-old male patient requiring permanent pacemaker implantation. *Conducted beat showing pre-excitation. **Conducted beat shows a normal PR interval.

There was no correlation between the PR interval and echocardiographic parameters; QRS duration correlated with maximal left ventricular wall thickness ($r = 0.4$, $p = 0.001$), LVMI ($r = 0.5$, $p < 0.001$), LVed ($r = 0.3$, $p = 0.003$) and LVes ($r = 0.3$, $p = 0.01$).

Clinical predictors of arrhythmia

All five patients with NSVT were male (mean age 58.4 $\pm$ 15.1 years, range 46-83). Three of the five patients with NSVT had symptoms of pre-syncope or syncope and all five had symptomatic palpitations. When compared to the rest of the cohort, the presence of syncope was associated with the presence of NSVT (OR = 24.5, 95% CI = 2.9, 207.3, $p = 0.006$), similarly the presence of palpitations was associated with NSVT (OR = 1.4, 95% CI = 1.04, 1.3, $p = 0.003$). This relationship was not present in patients with AF/PAF. Three of the five patients with NSVT had a QRS duration > 120ms. All five patients had a maximal left ventricular wall thickness ≥ 20mm and a LA size > 40mm (mean = 51.2 ± 9.7). The mean LVMI was 243.6 ± 102.3 gm/m^2 (range = 162.0 to 406.9). All the patients with NSVT had angiographically normal coronary arteries.

Univariate analysis identified age ($p<0.001$), LA size ($p = 0.001$), maximal left ventricular wall thickness ($p = 0.003$), LVMI (0.009) and a history of angina ($p = 0.016$) as predictors of AF and/or PAF. Stepwise logistic regression analysis identified age as the only independent predictor of atrial fibrillation (OR = 1.2, 95% CI = 1.1, 1.3, $p = 0.001$).

Table 2.2: Baseline Evaluation Indices in Males and Females

	All (n=78)	Female (n=35)	Male (n=43)	p-value
Age (years)	**43.5 ± 15.0**	**46.7 ± 15.6**	**40.7 ± 14.1**	**0.08**
Heart Rate (beats per minute)	**64.9 ± 14.8**	**67.2 ± 12.2**	**62.9 ± 16.4**	**0.2**
PR Interval* (ms)	**144.1 ± 26.5**	**149.3 ± 29.4**	**139.7 ± 23.2**	**0.12**
PR ≤ 120ms	**16 (21%)**	**5 (5%)**	**11 (27%)**	**0.36**
PR > 200ms (%)	**3 (4%)**	**2 (6%)**	**1 (2%)**	**0.36**
QRS Duration (ms)	**98.6 ± 19.7**	**90.1 ± 17.2**	**105.5 ± 19.0**	**<0.001**
QRS Duration >120ms	**12%**	**6%**	**16%**	**0.18**
Romhilt-Estes Score	**4.0 ± 2.9**	**2.4 ± 2.8**	**5.3 ± 2.2**	**<0.001**
Maximum LV Wall Thickness (mm)	**13.8 ± 4.7**	**11.7 ± 3.4**	**15.3 ± 5.0**	**<0.001**
Left Atrial Diameter* (mm)	**38.8 ± 6.7**	**37.2 ± 5.4**	**40.0 ± 7.5**	**0.07**
End Diastolic LV Diameter (mm)	**47.7 ± 5.4**	**45.5 ± 4.4**	**49.4 ± 5.6**	**0.001**
End Systolic LV Diameter* (mm)	**29.1 ± 4.9**	**28.2 ± 3.7**	**29.9 ± 5.6**	**0.08**
Fractional Shortening (%)	**39 ± 7**	**38 ± 7**	**39 ± 7**	**0.55**
LV Mass Index* (g/cm^2)	**134.7 ± 56.7**	**103.8 ± 23.8**	**158.9 ± 63.0**	**<0.001**

Table 2.2 Legend: Values expressed as mean ± standard deviation or %, * denotes variables with non-normal distribution, analysed using Wilcoxon Rank sum. LV = Left Ventricular.

Figure 2.2a: Age adjusted prevalence of arrhythmia in Anderson-Fabry Disease in Males

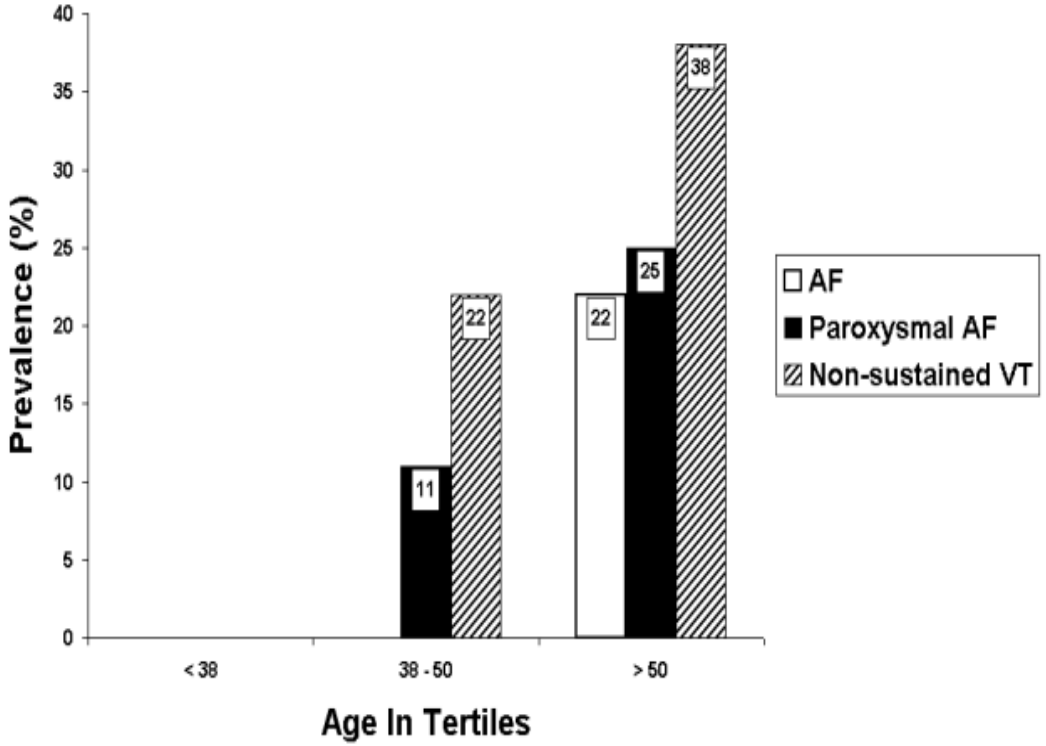


Figure 2.2a Legends: Age adjusted (age in tertiles) prevalence (%) of atrial fibrillation (AF), paroxysmal AF and non-sustained ventricular tachycardia (VT).

Figure 2.2b: Age adjusted prevalence of arrhythmia in Anderson-Fabry Disease in Females

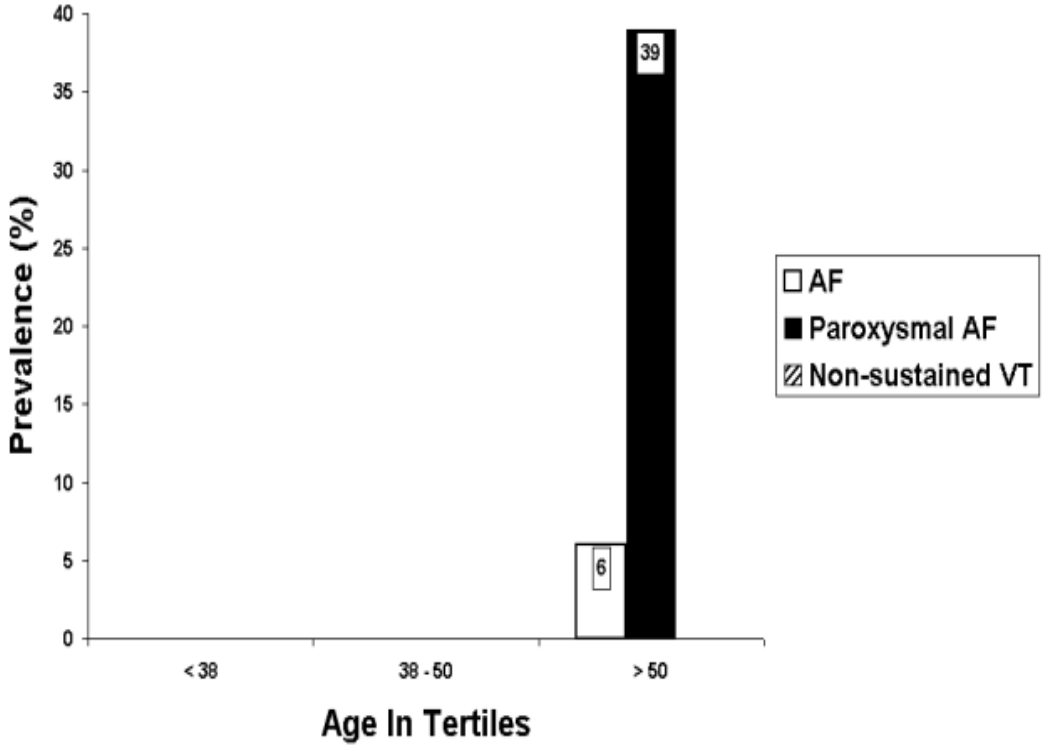


Figure 2.2b Legends: Age adjusted (age in tertiles) prevalence (%) of atrial fibrillation (AF), paroxysmal AF and non-sustained ventricular tachycardia (VT).

Follow-Up

Follow up data were available in 66/78 (84.6%) patients. The mean follow up time was 1.9 years (range = 3 months – 10 years). During follow up, 7/66 (10.6%) patients had permanent pacemakers implanted; 1 for complete heart block; (patient with pre-excitation, Figure 2.1); 3 for symptomatic bradycardia; 1 for symptomatic LV outflow tract gradient reduction; 1 for complete heart block post alcohol septal ablation (the latter two patients had been followed-up with a presumed diagnosis of hypertrophic cardiomyopathy for several years prior to the diagnosis of AFD); 1 had a bi-ventricular device and internal cardioverter defibrillator for heart failure and symptomatic NSVT (Figure 2.3).

There were 2/66 (3.0%) new cases of persistent AF, one patient with persistent AF at baseline evaluation had been successfully cardioverted to sinus rhythm and one remained in permanent AF. There were 2 new cases of PAF documented.

Discussion

This study shows that arrhythmia is common in patients with AFD and that it is associated with significant morbidity.

Population based studies have shown that the overall prevalence of atrial fibrillation in the normal population is less than 1% [86]; this prevalence is age dependent, with a prevalence of less than 1% in people less than 55, rising to 9% or more in the over eighties [86].

Figure 2.3: Non-Sustained Ventricular Tachycardia

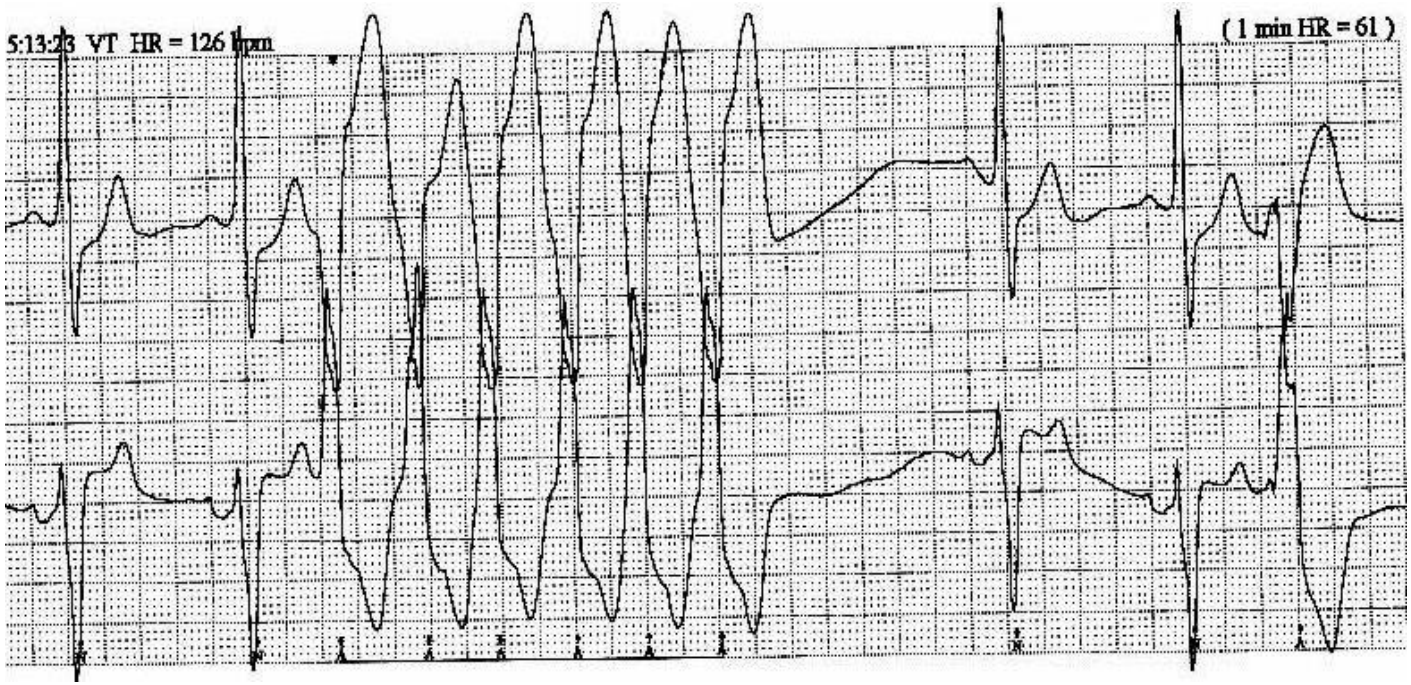


Figure 2.3 legend: Twenty-four hour ambulatory ECG recording of symptomatic 6 beat non-sustained ventricular tachycardia in a 59-year-old male patient requiring internal cardioverter defibrillator implantation.

In this study, the overall prevalence of AF was four times higher than in the general population; in the over fifties it was present in 12%. This suggests that AFD is associated with a substantial risk of AF in middle aged and elderly patients.

Gender related differences

Although AFD is an X-linked disorder, most females who carry a pathogenic mutation in the α-Gal gene develop signs and symptoms of the disease, albeit at an older age than men [68]. A striking observation in this study was that, in spite of a higher incidence of "classical" signs and symptoms such as angiokeratomata, neuropathic pain and hypohidrosis in men, the incidence of cardiovascular symptoms was similar in males and females. Men tended to have a longer QRS duration and higher Romhilt-Estes score than women, reflecting their greater left ventricular mass. Similarly, all the patients with NSVT were men; there was, however, no gender related difference in the frequency of AF, the only independent risk factor being age. It is likely, therefore, that most patients with AFD become prone to cardiac disease and arrhythmia if they live into middle age and beyond.

Clinical Implications

The impact of arrhythmia on mortality in patients with AFD cannot be determined from this study, but the high prevalence of atrial fibrillation, the occurrence of complete heart block and symptomatic ventricular tachycardia, suggests that it may contribute to the shortened life expectancy of patients with this disease. This observation suggests that all patients with AFD should undergo regular assessment with ECG and Holter monitoring.

Patients with AFD have a higher incidence of stroke that is usually attributed to microvascular dysfunction[87-89]. Additionally, an increased thrombotic potential, dilative arteriopathy and changes in regional cerebral hyperperfusion may contribute to cerebrovascular disease in patients with AFD[49-51]. Lenders *et al* suggest that up to 7% of patient with AFD also carry a mutation in the Factor V Leiden gene. These patient have a 5 fold increase (95% CI HR = 2-13) in the risk of suffering a thromboembolic event[90]. The high prevalence of atrial arrhythmia in this study, suggests that thromboembolism may be an additional risk factor for stroke, and consideration of anticoagulation in patients with permanent or frequent paroxysms of AF may be needed. Unfortunately, the early age of onset of atrial arrhythmia compared to the general population precludes the use thromboembolism risk scores such as the CHA_2DS_2-VASc scoring system in this patient group[91].

There has been no systematic study of sudden death in AFD. To date, there have been four case reports published describing the unexpected death of patients with AFD. Three were asymptomatic elderly females (diagnosed at post-mortem) [92, 93], one was a 26-year-old male known to have AFD who died whilst running [94]; and one was a male known to have AFD who had ventricular fibrillation resistant to defibrillation [95]. Three of the four patients had post-mortem evidence of significant cardiomyopathy with hearts weighing >450gms. All the patients with NSVT in our study had evidence for significant cardiac disease (all male, increased LVMI and maximal left ventricular wall thickness > 20mm). Together these data suggest that sudden death in AFD is associated with significant cardiac involvement and may be related to NSVT.

Conclusions

This study demonstrates that arrhythmias are common in older patients with AFD. This, together with the high prevalence of permanent pacemaker implantation, suggests that there is a need for regular cardiology follow-up in this patient group, monitoring for rhythm abnormalities.

Chapter 3

The natural history of left ventricular systolic performance

Abstract

Aim

Although AFD cardiomyopathy is usually associated with left ventricular hypertrophy, conduction disease and valvular thickening, a recent study has demonstrated an impairment of contractile function as well. The aim of this study was to determine the importance of systolic function in the natural history of AFD.

Methods and results

Twelve patients 9 male, (aged; 54.5 ± 12.2) years; minimum = 42 and maximum = 82) with AFD were studied. All patients underwent clinical, electrocardiographic and echocardiographic evaluation. Two echocardiograms >1 year apart were performed on all patients. Patients on enzyme replacement therapy (ERT) were excluded from the study.

The mean follow up between echocardiograms was 3.3 ± 2.7 years (minimum = 1, maximum = 9). There was no change in left ventricular mass. There was an increase in the end systolic left ventricular diameter of 5.0mm (95% confidence interval (CI) = 0.2, 9.6, p = 0.038) and a decrease in fractional shortening of 6.1% (95% CI = 0.3, 11.9, p=0.042). Three patients (25%) developed symptomatic congestive heart failure.

Conclusions

Systolic impairment in patients with AFD cardiomyopathy is common. The decline in systolic function may represent a measure of disease severity and provide a clinically

important surrogate marker for response to therapy.

Aims

The aim of this study was to determine the importance of systolic performance in the natural history of AFD and its potential value as marker of disease progression, we performed a retrospective analysis of an untreated cohort of patients followed for at least one year.

Methods

A total of 75 patients with AFD recruited from The Heart Hospital, London, The Royal Free Hospital and The National Hospital for Neurology and Neurosurgery, were evaluated between 1st January 1993 and 1st December 2003. Diagnosis was based on low plasma α-Gal levels and on genetic mutational analysis. The mean (standard deviation) follow up from diagnosis of AFD was 5.8 ± 4.8 years. Of the 75 patients 24 had been followed up for more than one year and 12 were receiving ERT at the time of evaluation. The final study cohort comprised 12 untreated patients (9 male, 3 female patients mean age 54.5 ± 12.2 years; minimum age = 42, maximum = 82) with at least 2 echocardiograms separated by one year or more. Six patients were detected during screening of unexplained hypertrophic cardiomyopathy (HCM)[79], the remaining six were patients referred for cardiac assessment of patients with AFD.

Clinical assessment

All patients underwent clinical examination, supine 12-lead electrocardiography (ECG) (Hewlett-Packard, USA) and 24 hour ambulatory ECG (Reynolds Medical, UK) monitoring as described in chapter 2.

Plasma α-Gal analysis

Plasma α-Gal activity was measured with the fluorogenic substrate 4-methylumbelliferyl-α-Dgalactopyranoside (Sigma), with N-acetyl-D-galactosamine (Nacalai Tesque) used as an inhibitor of α-N-acetylgalactosaminidase as described previously [96]. On the basis of previously published data, a plasma α-Gal activity of 1.2 $nmol \cdot h^{-1} \cdot mL^{-1}$ was considered diagnostic of AFD [96]. All patients with diagnostic plasma α-Gal levels went on to have confirmation of their diagnosis with genetic mutational analysis.

Assessment of left ventricular mass and function

M-mode, 2D, and Doppler echocardiographic assessment of all patients was as previously described in chapter 2 using a GE System V echocardiograph.

Statistical analysis

Statistical analysis was performed using SPSS v19.0 (Chicago, USA). The χ^2 test was used to compare non-continuous variables, and the 2-tailed paired sample Student's *t*-test was used to compare continuous variables. All values are expressed as means ± standard deviation. Statistical significance was defined as p 0.05.

Results

Table 3.1 describes the baseline symptoms and echocardiographic findings in this cohort. The mean time between the analysed echocardiograms was 3.3 ± 2.7 years (minimum = 1, maximum = 9).

Four patients (41%) had two symptoms or signs of AFD, and one in retrospect had 4

symptoms or signs of AFD. Ten patients (83%) presented with a cardiovascular symptom. Six patients (50%) had two or more cardiac symptoms (see table 3).

During the follow up, 2 (17%) patients had documented non-sustained ventricular tachycardia; one (8%) had an internal cardiac defibrillator implanted prophylactically for this. Four (33.3%) patients required permanent pacemaker implantation: two for symptomatic bradycardia; one for congestive heart failure (bi-ventricular device); and one patient for complete heart block following alcohol septal ablation prior to the diagnosis of AFD. Two other patients progressed to congestive heart failure.

Electrocardiography

The baseline mean P-R interval was 141.5 ± 22.3 ms with a baseline mean QRS duration of 112.7 ± 25.0 ms. During follow up, no difference in the P-R interval was seen. There was an increase in the QRS duration (mean = 14.2ms; 95% CI = 4.0, 24.4ms, p = 0.012). Patients with pacemakers were excluded from this analysis.

Echocardiography

Three patients (25%) had normal left ventricular wall thickness, eight (66.7%) had concentric LVH and one patient (8.3%) had distal LVH. Figures 3.1 & 3.2 plot the changes in LVes, LVed and FS in individual patients. There was an increase in the LVes (mean 5 ± 6.9 mm, 95% CI = 0.2, 9.6, p=0.038), accompanied by a reduction in the FS (mean 6.1 ± 8.7 mm, 95% CI = 0.3, 11.9 mm, p=0.042) and a trend towards an increasing LVed (mean 2.9 ± 5.4, 95% CI = -0.5, 6.35 mm, p=0.088). The rate of dilatation in the left ventricular end systolic dimension was 1.9mm/year with an associated decline of 2.7 %/year in the fractional shortening. There was no correlation between age and baseline LVes.

Table 3.1: Anderson-Fabry Disease Symptoms and Baseline Echocardiographic Findings

SEX	AGE	Mutation	Plasma α-Gal Level	Leukocyte α-Gal Activity	Angina	Dyspnoea	Syncope	Palpitations	Maximum LV Wall Thickness (mm)	LV End Systolic Dimension (mm)	LV End Diastolic Dimension (mm)	Fractional Shortening (%)
M	46	Asn215Ser	0.99	7.4	-	1	-	+	24	30	52	42
M	82	Asn215Ser	0.25	4.7	+	2	-	+	26	25	38	34
F	75	Deletion	4.30	84.0	-	1	-	-	11	25	41	39
M	50	Asn215Ser	0.28	5.4	+	2	-	+	24	19	35	46
M	58	Ile317Thr	0.04	5.2	+	2	-	+	21	33	50	34
F	45	520delT	1.6	N/A	+	1	-	-	10	30	50	40
M	53	Deletion	0.64	1.8	-	1	-	+	20	27	53	49
M	49	Asn215Ser	0.77	7.8	+	2	-	-	14	26	44	41
M	46	Arg301Gln	0.10	3.6	-	1	+	+	20	21	43	51
M	55	Ile317Thr	0.40	3.1	-	1	-	-	24	31	50	38
F	53	Deletion	3.90	63.0	+	2	-	-	11	24	45	47
M	42	Gly361Arg	0.31	2.2	-	2	-	-	24	21	46	54

Table 3.1 legend: α-Gal = α-Galactosidase, Angina = Exertional chest pain, Dyspnoea expressed as New York Heart Association dyspnea class, LV = Left Ventricular.

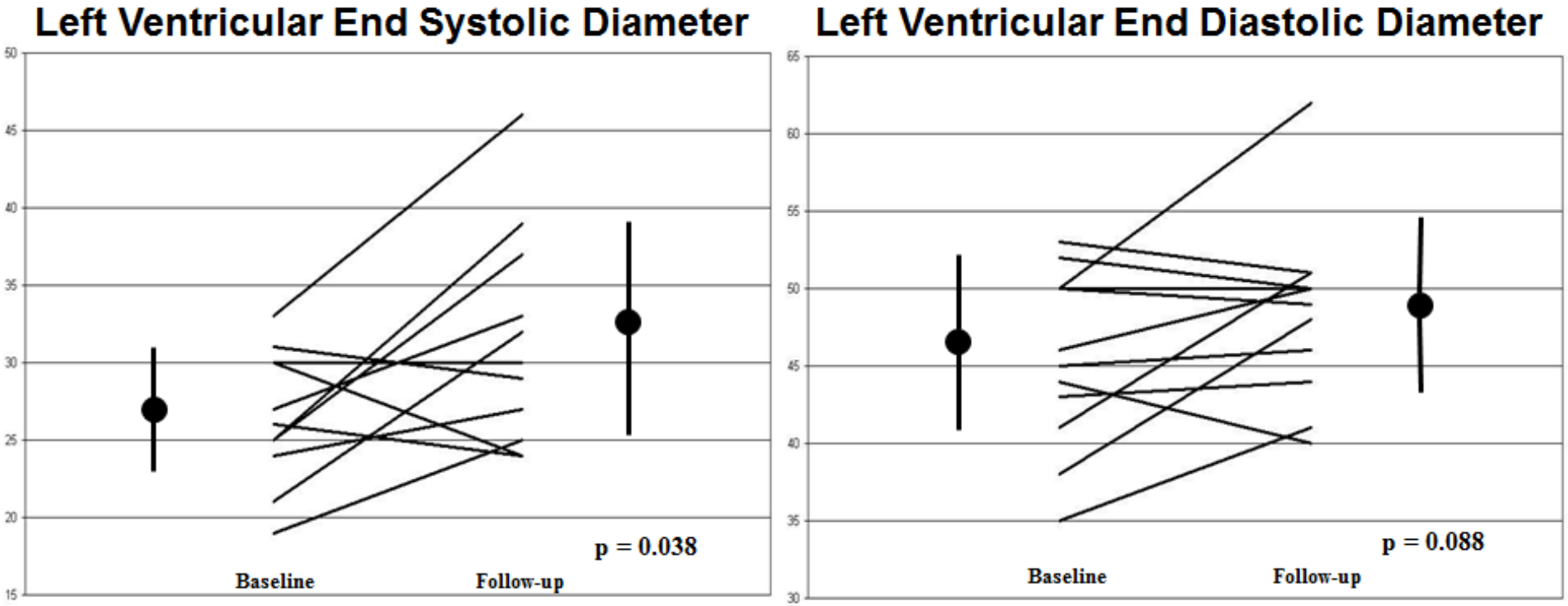
There was a negative correlation between plasma (and leukocyte) α-Gal and baseline maximal LV wall thickness and LV mass index ($r = -0.6$, $p = 0.045$; $r = -0.6$, $p = 0.033$ respectively). There were no correlations between α-Gal levels and baseline LV cavity dimensions. Similarly there were no correlations between enzyme levels and any changes in these dimensions during follow up.

Three patients demonstrated a reduction in LVes during follow-up. One patient had a 6mm reduction in the LVes dimension and an 11% increase in the FS. This patient was a 45 years old female. During follow up, she developed a 5mm increase in LV wall thickness and a change in her LV geometry from normal to concentric LVH. There were no changes to medications or in symptoms. The other two patients had 2mm reductions in LVes. These reductions were associated with a 1% reduction in FS in one patient and an increase of 4% in the other.

The baseline mean RWT was 0.71 (0.21) (range 0.38-1.16). One patient (female) had normal LV geometry at baseline, eight (66.7%) had concentric LVH and three (25%) had concentric remodeling. Of the three patients with concentric remodeling, one progressed to concentric LVH; one to normal geometry and one had no change on follow up. Of the 8 patients with concentric LVH at baseline, 1 patient regressed to concentric remodeling, 1 patient developed eccentric LVH and 6 patients remained unchanged at follow up.

Figure 3.1: The Change in Left Ventricular End Systolic Diameter, and Left Ventricular End Diastolic Diameter During Follow Up

Figure 3.1 legend: Error bars represent mean (SD) for baseline and follow-up respectively: Left Ventricular End Systolic Diameter (mm); 26.5(4.4), 31.5(7.0): Left



Ventricular End Diastolic Diameter (mm); 45.6(5.7), 48.5(5.7).

Figure 3.2: The Change in Fractional Shortening During Follow Up

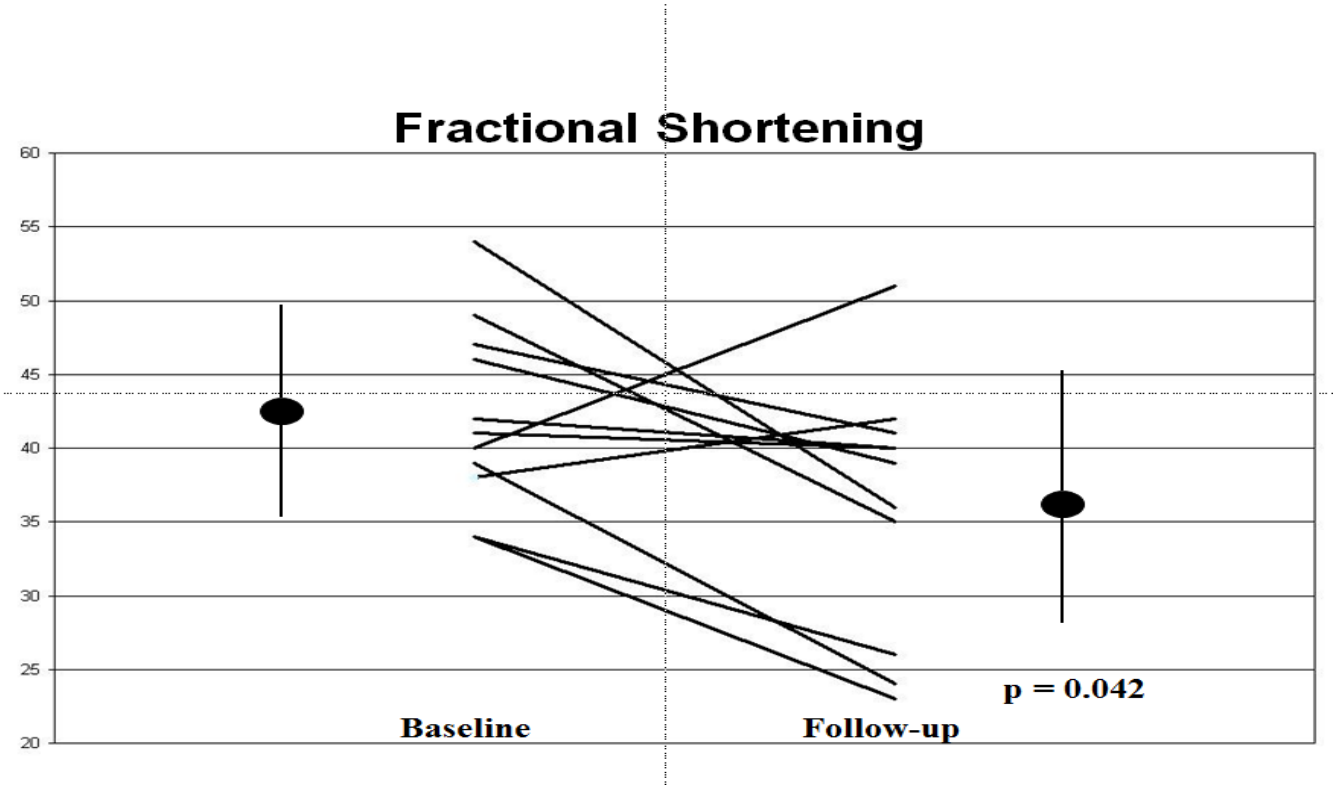


Figure 3.2 legend: Error bars represent mean (SD) for baseline and follow-up respectively: Fractional Shortening (%); 42.9(6.5), 36.1(8.6).

Discussion

This study demonstrates that left ventricular systolic function progressively deteriorates in patients with AFD. Importantly, the change in systolic performance was not accompanied by a change in left ventricular mass index. This suggests that systolic function may be a sensitive surrogate marker of disease severity and prognosis in patients with AFD cardiomyopathy.

Cardiac disease in AFD

The most commonly reported cardiac abnormalities in patients with AFD include increased QRS voltage and repolarisation changes, short PR interval, QRS prolongation, concentric left ventricular hypertrophy and valvular thickening [77, 97, 98]. Previous isolated case reports have reported congestive cardiac failure from restrictive cardiomyopathy and LV dilatation, with one case requiring cardiac transplantation[62, 99-103].

To the best of our knowledge, this is the first time that the deterioration in systolic dysfunction and progression to congestive cardiac failure has been reported in a prospectively studied cohort of patients with AFD.

Mechanism of systolic dysfunction

Although the genetic and biochemical basis of AFD is well described, the pathophysiology of cardiac disease in AFD is inadequately understood. Histologically, AFD is characterised by myocyte vacuolation and intralysosomal inclusions on electron microscopy [97]. However, the consequences of these abnormalities on myocyte function are not known. Moreover, some data suggest that substrate accumulation

accounts for only 3% of the increased LV mass seen in AFD patients [62]. Patients with AFD have areas of interstitial expansion on gadolinium enhanced cardiac magnetic resonance imaging [63]. It is unclear whether this is a primary or secondary phenomenon, but the presence of interstitial abnormalities suggest that progressive fibrosis may also contribute to the reduction in systolic performance observed in this study.

Clinical implications and limitations

Our study suggests that systolic function may be a surrogate marker of disease severity in AFD. Weidemann et al have recently shown that enzyme replacement therapy using a recombinant α-galactosidase A preparation improves longitudinal and radial strain rate in patients with AFD [74]. Strain rate imaging is a new technique that quantifies changes in regional myocardial deformation, and is thought to be a sensitive measure of myocardial function [104]. Our findings and Weidemann *et al* study support the hypothesis that systolic function may also be a useful surrogate end-point with which to assess the response to treatment. This study is limited by the small size of the cohort and the relatively simple assessment of systolic function.

Conclusions

This study suggests that cardiac systolic dysfunction is common in patients with AFD and hence systolic performance should be monitored in all patients with AFD.

Chapter 4

Exercise capacity

Abstract

Background

Patients with AFD frequently complain of exercise limitation, but few studies have quantified its severity and relation to disease severity.

Methods and Result

Cardiopulmonary exercise testing (CPET) was performed in 103 consecutive patients (mean age 43.2±14.8 years, 53 (51.5% male) with AFD; the relation between gas exchange parameters, cardiovascular manifestations and other markers of disease severity was determined. Oxygen consumption at peak exercise (pVO_2) was less than 80% of predicted maximum ($\%VO_2max$) in 51 patients (33 (64.7%) male, 18 (35.3%) female; p=0.01). Males had lower % predicted VO_2max (p=0.001), % predicted peak work rate (p<0.001), and anaerobic threshold (p=0.04) when compared to females. Stepwise linear regression identified Mainz severity score index (MSSI) score as the only independent predictor of % predicted VO_2 max (β=-1.02, p<0.001).

Conclusions

Males with AFD have more severe exercise limitation when compared to females. The utility of pVO_2 as a surrogate marker of disease severity warrants further investigation.

Aim

Many patients with AFD die prematurely in the fourth and fifth decade of life from stroke, heart failure and renal failure, but throughout life, experience fatigue and exercise limitation [105]. The aim of this study was to determine exercise capacity using

symptom limited cardiopulmonary exercise testing (CPET) in a large referral population of patients with AFD and its relation to markers of disease severity.

Methods

One hundred and eleven consecutively referred patients (mean age 43.0±15.6 years; 56 (50.5%) males) with AFD were assessed at the Heart Hospital, University College Hospitals, London, between January 1993 and February 2005. The diagnosis of AFD was based on plasma A-Gal levels and mutational analysis. Sixty-two patients (55.9%, 44.9 (12.4) years, 45 (81.8%) males) were receiving enzyme replacement therapy. All patients underwent clinical history and physical examination. The following cardiac symptoms were noted: angina (exertional central chest pain lasting ≤15 minutes); dyspnoea (graded using New York Heart Association criteria, NYHA); syncope and palpitation.

All patients underwent clinical examination, supine 12-lead electrocardiography (ECG) (Hewlett-Packard, USA) and 24 hour ambulatory ECG (Reynolds Medical, UK) monitoring as described in chapter 2.

Mainz severity score index (MSSI)

In brief, the MSSI scoring system comprises four components: general, neurological, cardiovascular, and renal[106]. Each component consists of a group of signs and symptoms associated with AFD, weighted according to their contribution to morbidity. For each sign and symptom, a score is assigned and summed to produce a total score

for that system component. These individual component scores are then totaled to give the final MSSI score (Table 4.1).

Cardiopulmonary testing

Patients were exercised in the upright position using a bicycle ergometer (Ergometrics 800S, SensorMedics, Inc., Yorba Linda, California, USA) with continuous 12-lead electrocardiographic monitoring (Max 1, Marquette Electronics Inc., Milwaukee, Wisconsin).

Breath-by-breath respiratory gas sampling was performed using a VMax 229 Console, (SensorMedics, Inc). Patients were exercised using a 10 to 25 W/min incremental ramp protocol adjusted to patients' subjective assessment of their exercise capacity. Patients cycled at a constant rate of 60 to 65 rpm to the point of maximum effort. Respiratory gases were sampled continuously from a mouthpiece and analyzed using a 1111D/000 paramagnetic transducer for oxygen and a 2900 MMC non-dispersive infrared sensor for carbon dioxide.

Table 4.1 : Mainz Severity Score Index

Mainz Severity Score Index							
General		Neurological		Cardiovascular		Renal	
Symptom/Sign	Score	Symptom/Sign	Score	Symptom/Sign	Score	Symptom/Sign	Score
Facial appearance	0/1	Tinnitus	0/1/2	LVH	0/1/6/8/12	Renal involvement	0/4/8/12/18
Angiokeratoma	0/1/2	Vertigo	0/1/2	Valve abnormalities	0/1		
Oedema	0/1	Acroparaesthesiae	0/3/6	ECG abnormalities	0/2		
Musculoskeletal	0/1	Pyrexia pain crisis	0/2	Pacemaker	0/4		
Cornea verticillata	0/1	Cerebrovascular	0/1/3/5	Hypertension	0/1		
Sweating	0/1/2	Depression	0/1				
Abdominal Pain	0/2	Fatigue	0/1				
Altered Bowel	0/1	Reduced activity level	0/1				
Piles	0/1						
Airways disease	0/2						
NYHA	0/1/2/3/4						
Max Score 18		Max Score 20		Max Score 20		Max Score 18	

Table 4.1 legend: Adapted from Whybra et al [106]. NYHA = New York Heart Association dyspnoea class; LVH = left ventricular hypertrophy, points for ECG and echocardiographic evidence of LVH; Neurological component has points for computed tomography / magnetic resonance imaging abnormalities, history of transient ischemic attack; Renal component includes reduced glomerular filtration rate / creatinine clearance and end stage renal failure and dialysis.

The signals underwent analogue to digital conversion for the calculation of oxygen consumption (VO_2) and carbon dioxide output (VCO_2) using an established technique[107, 108]. A printout of VO_2 (l/min), VCO_2 (l/min), heart rate (HR) (beats per min), work rate (W) and respiratory quotient (RQ) was obtained and averaged at 10 s intervals to obtain smooth graphical representation. Plots of VCO_2 against VO_2 were used to estimate anaerobic threshold (AT). Blood pressure was recorded at 2-minute intervals during exercise and for 10 minutes into recovery using a brachial cuff and sphygmomanometer. Change in blood pressure (defined as peak exercise SBP – pre-exercise SBP) was recorded. All tests were supervised by an experienced senior physiologist.

The following calculations were made:

1. Peak oxygen consumption (pVO_2) was defined as the highest oxygen consumption achieved during exercise. This was the highest measured VO_2 value over the last 10 s of exercise. Data were presented as values normalized for body weight (ml/kg/min). The predicted maximal oxygen consumption (VO_2 max) was calculated using established equations based on age and gender[109, 110]. The normalized value was then expressed as a percentage of the predicted maximum value. Values <80% were considered abnormal because they fall below previously established 95% confidence limits [111].

2. Anaerobic threshold is the VO_2 above which aerobic energy production is supplemented by anaerobic mechanisms and is reflected by an increase in lactate and lactate/pyruvate ratio in the muscle and arterial blood [111] [21]. This can be estimated

noninvasively by plotting VCO_2 versus VO_2 ("V slope method"; [112]. The AT was expressed as a percentage of the predicted VO_2 max. Values<40% predicted VO_2 max were considered abnormal [112].

3. Oxygen pulse (O_2P) is the ratio of VO_2 to heart rate (HR) (equation 1). It is the product of stroke volume (SV) and the systemic arteriovenous oxygen difference (A-V) O_2 (equation 2).

$O_2P=VO_2/HR$ (1)

VO_2 max=Cardiac output×(A-V) O_2

Cardiac output=SV×HR

$\therefore O_2P=SV\times(A\text{-}V)\ O_2$. (2)

Values were expressed as a percentage of the maximum predicted O_2P calculated from equation 1 using established formulae for predicted VO_2 max [109, 110] and predicted maximum heart rate [113, 114]. Values >80% predicted VO_2 max were regarded as normal if the individual had achieved a maximal heart rate of at least 80% to exclude chronotropic incompetence, because this is associated with falsely high readings [113, 114].

Statistical analysis

Statistical analysis was performed using SPSS v19.0 (SPSS, Inc., Chicago, Illinois, USA). Analyses were stratified for gender. The chi-square test was used to compare non-continuous variables and the two-tailed, independent-samples student t test was used to compare continuous variables. All values were expressed as mean ± SD. Stepwise linear regression analysis was used to identify independent predictors of pVO2 using

those variables significantly associated with pVO2 in a univariate analysis. Statistical significance was defined as $p < 0.05$.

Results

CPET data were unavailable in 8 (7 %) of the 111 patients. The reasons were as follows: 3 patients were less than 16 years of age (all female); 1 was 80 years of age (female); 2 were wheelchair bound (of which one was male); 1 had fast atrial fibrillation at presentation (male) and 1 refused testing (female).

Baseline characteristics of the final study cohort (n=103) are shown in Table 4.2. Males had a higher prevalence of AFD symptoms and higher MSSI scores compared to females (mean difference = 13.0, 95% CI = 8.5, 17.4, $p < 0.001$). Males tended to have higher levels of proteinuria and lower glomerular filtration rates when compared to females (Table 4.2).

Cardiac disease

Cardiac findings in males and females are summarized in Table 4.3. There was no difference in the prevalence of cardiovascular symptoms between males and females. Males tended to have lower resting heart rates compared to females (mean difference = 5.5 bpm, 95% CI = -0.1, 11.1; $p = 0.05$). The QRS duration was greater in males compared to females (mean difference = 15.7 ms, 95% CI = 8.4, 23.0; $p < 0.001$). Males had a higher RE score compared to females (mean difference = 2.6, 95% CI = 1.5, 3.7; $p < 0.001$, Table 4.2).

Table 4.2: Anderson - Fabry Disease Symptoms and Signs Stratified By Gender

	Females (n = 50)	Males (n = 53)	Significance
Age (Years)	45.4 (15.4)	41.2 (14.1)	0.2
Plasma A-Gal levels (nmol/hr/ml)	3.8 (1.4)	0.6 (0.9)	<0.001
Enzyme replacement therapy (%)	16 (27.1)	43 (79.3)	<0.001
MSSI score	18.5 (10.3)	31.5 (11.9)	< 0.001
Acroparasthaesia (%)	23 (46.0)	42 (64.6)	0.002
Hypohidrosis (%)	13 (26.0)	27 (50.9)	0.02
Abdominal pain (%)	16 (32.0)	23 (43.4)	0.4
Tinnitus (%)	5 (10.0)	20 (37.7)	0.002
Angiokeratomata (%)	7 (14.0)	34 (64.2)	<0.001
Glomerular filtration rate (ml/min/m^2)	89.3 (23.8)	80.8 (27.5)	0.2
Proteinuria (g/24hr)	0.2 (0.2)	0.3 (0.4)	0.1

Males had greater maximal left ventricular wall thickness ($p<0.001$), greater left ventricular dimensions ($p<0.001$ for LVed and $p=0.04$ for LVes) and greater left ventricular mass index ($p<0.001$).

Exercise capacity in males and females

Metabolic exercise data for males and females are shown in Table 4.4. Males had a lower peak exercise oxygen consumption compared to females (mean difference = 16.1% 95% CI = 7.0, 25.3; p = 0.001).

There was a trend for more males to have an abnormal pVO_2 (< 80%) compared to females (33 (62.3%) versus 18 (36%) respectively; RR = 1.7, 95% CI = 1.5, 2.5; p = 0.01). There were no differences in pVO_2 in patients receiving ERT compared to those not receiving ERT. The amount of work performed by males during exercise was less when compared to females (mean difference = 19.6%, 95% CI = 9.3, 29.9; p < 0.001) despite similar respiratory quotients. Males had a lower anaerobic threshold compared to females (mean difference = 3.9, 95% CI = 0.2, 7.7; p = 0.04); the number of males and females with abnormally low anaerobic thresholds was similar (25 (52.1%) versus 16(34.8%), respectively; p = 0.1). More men than women had a peak HR response of less than 80% of their predicted value at peak exercise, 30 (56.6%) versus 18 (36.0%), RR = 1.5, (95% CI = 1.0, 2.1; p = 0.048).

Despite the lower peak exercise heart rates, males had a lower oxygen pulse compared to females (mean difference = 10.3%, 95% CI = 0.6, 20.0; p = 0.04).

Table 4.3: ECG and Echocardiographic Data Stratified By Gender

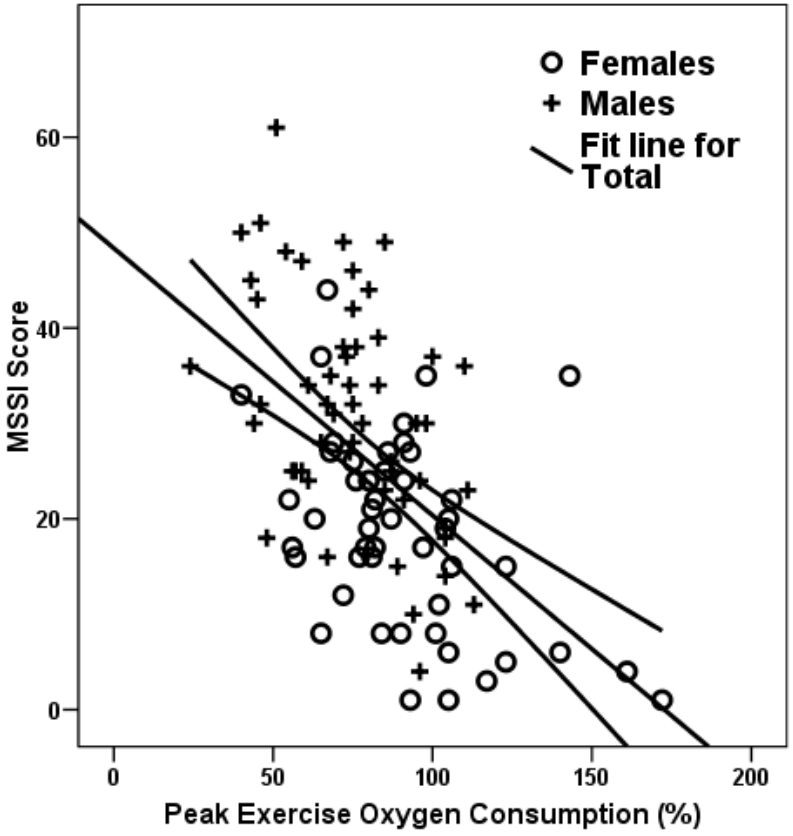
	Females (n = 50)	Males (n = 53)	Significance
Cardiac symptoms			
Angina (%)	9 (18.0)	10 (18.9)	1.0
Dyspnoea (NYHA ≥ 2, %)	9 (18)	17 (32.1)	0.2
Palpitations (%)	13 (26.0)	13 (24.5)	1.0
Syncope (%)	3 (6.0)	4 (7.5)	1.0
ECG Data			
Resting heart rate (beats/minute)	67.3 (12.7)	61.8 (15.0)	0.05
PR interval (ms)	150.9 (28.1)	142.9 (22.8)	0.1
QRS duration (ms)	90.4 (18.2)	106.1 (18.4)	<0.001
Romhilt-Estes Score	u2.9 (3.1)	5.5 (2.5)	<0.001
Echocardiographic Data			
Maximal LV wall thickness (mm)	11.2 (2.7)	15.0 (4.5)	<0.001
Left atrial size (mm)	36.5 (4.9)	40.6 (7.5)	0.002
LV end diastolic diameter (mm)	46.0 (4.0)	50.1 (5.6)	<0.001
LV end systolic diameter (mm)	28.4 (3.6)	30.4 (5.6)	0.04
Fractional shortening (%)	38.4 (6.0)	39.5 (6.7)	0.3
LV mass index (g/cm^2)	99.5 (25.3)	147.1 (48.9)	<0.001

Table 4.4: Exercise Data Stratified By Gender

	Females (n = 50)	Males (n = 53)	Significance
Peak exercise oxygen consumption (%)	90.5 (26.0)	74.4 (20.8)	0.001
Work (%)	83.4 (23.8)	63.8 (27.3)	<0.001
Anaerobic threshold (%)	44.2 (9.2)	40.3 (9.0)	0.04
Heart rate at peak exercise (%)	82.2 (12.6)	75.9 (15.2)	0.02
Oxygen pulse (%)	110.2 (25.9)	99.8 (22.7)	0.04
Respiratory quotient	1.0 (0.1)	1.0 (0.1)	0.8
Δ blood pressure at peak exercise (mmHg)	45.7 (15.6)	57.2 (23.0)	0.004
Exercise time (minutes:seconds)	07:42 (01:24)	08:01 (02:37)	0.5

Table 4.4 legend: % = percent of predicted maximum

Figure 4.1: Correlation between percentage predicted VO2 max and MSSI score



Males had a larger increase in systolic blood pressure during exercise compared to females (mean difference = 12.0mmHg, 95% CI = 4.2, 19.7; p = 0.003).

Clinical correlates of pVO_2

There was no relation between pVO_2 and a history of chest pain, palpitations or syncope. Patients with dyspnoea (NYHA class ≥ II) had a lower pVO_2 compared to those without (mean difference = 16.4%, 95% CI = 5.6, 27.2; p = 0.003). There was a negative correlation between percentage predicted VO2 max and MSSI score (Figure 4.1; r = - 0.5, p < 0.001). There was no correlation between percentage predicted VO_2 max and the degree of proteinuria (quantified through 24 hour urine collection*)* or GFR (measured using EDTA nuclear medicine techniques).

There was a negative correlation between percentage predicted VO_2 max and the QRS duration on ECG and the RE score (r = - 0.3 and r = - 0.3; p = 0.006 and p = 0.001 respectively). There were significant correlations between percentage predicted VO_2 max and maximal LV wall thickness, left atrial size and LV mass index (r = - 0.2, - 0.3, - 0.2; p = 0.04, 0.007, 0.02 respectively)**.**

To identify the clinical predictors of percentage predicted pVO_2, a stepwise regression analysis model was used. Co-variates entered into the model were those that were identified as being significantly associated with percentage predicted VO_2 max; namely: gender, NYHA class ≥ II, MSSI score, QRS duration, RE score, maximal LV wall thickness, LA size and LV mass index. Using this model the only independent clinical predictor of percentage predicted VO_2 max was MSSI score (β = - 1.0, 95% CI = - 0.6, -1,4; p < 0.001).

Discussion

This study shows that the majority of men and a substantial minority of women with AFD have objective evidence for exercise limitation. The degree of impairment correlates with overall disease severity.

There are many potential mechanisms of exercise limitation in patients with AFD. Glycosphingolipid accumulation in cardiomyocytes and myocardial fibrosis are common in adults with AFD[115, 116]. By contributing to abnormal myocardial contractility and diastolic dysfunction, these abnormalities would be expected to impair stroke volume response to exercise and result in elevated left atrial pressure, thereby leading to pulmonary congestion and exertional dyspnea. In this study, many patients had diminished heart rate responses which contributed to a reduced cardiac output at peak exercise. The mechanism of this chronotropic incompetence is unknown, but it probably reflects the effect of Gb3 accumulation in the sinoatrial node and specialized conduction system of the heart [117] as well as impaired autonomic function [118]. Valvular disease is also common in patients with AFD, and could theoretically contribute to exercise limitation if severe [61].

In this study a number of exercise, ECG and echocardiographic parameters were significantly worse in males compared to females. This would be consistent with more severe disease manifestations in hemizygous males compared to heterozygous females. There was however no significant difference in cardiac symptoms between males and females which is consistent with previous data[119].

Mild airflow obstruction is common in patients with AFD [120]. It is similar to the fixed obstruction that is seen in COPD with infiltration of inflammatory cells, smooth muscle hyperplasia, uncoupling of smooth muscle from the adventitia, bronchial wall hypertrophy and fibrosis being potential mechanisms [120]. This was not quantified in this study but may have impacted on pVO_2.

The percentage of predicted VO_2 max correlated significantly with MSSI score, ECG parameters (RE score and QRS duration) and echocardiographic parameters (maximal LV wall thickness, left atrial size and LVMI). However, the only independent predictor of pVO_2 was found to be MSSI score, This is perhaps not surprising given that both MSSI and pVO_2 take account of multiple organ systems affected by AFD, but suggests that cardiopulmonary exercise testing may be a useful method for assessing disease severity and perhaps the response to enzyme replacement therapy.

In conclusion, objective impairment of exercise capacity is common in patients with AFD and may be a useful marker of disease severity which deserves further investigation.

Chapter 5

Coronary microvascular function, its relation to symptoms and response to enzyme replacement therapy

Abstract

Background

In chapter 3, I described how patients with AFD develop progressive systolic dysfunction. AFD cardiomyopathy is also associated with myocardial fibrosis. These phenomena could be secondary to coronary microvascular dysfunction. Aim of this study was to measure coronary flow reserve (CFR), an index of microvascular function, in AFD at baseline and after enzyme replacement therapy (ERT).

Methods and results

Myocardial blood flow (MBF) at rest and during hyperaemia (adenosine 140 μg/kg/min) was measured in 10 male, non-smoking patients (53.8±10.9 years, cholesterol 5.5±1.3 mmol/l) and 24 age matched male, non-smoking controls (52.0±7.6 years, cholesterol 4.5±0.6 mmol/l) using positron emission tomography (PET). Resting and hyperaemic MBF and CFR (hyperaemic/resting MBF) were reduced in patients compared to controls (0.99±0.17 vs. 1.17±0.25 ml/g/min, $p<0.05$; 1.37±0.32 vs. 3.44±0.78 ml/g/min, $p<0.0001$ and 1.41±0.39 vs. 3.03±0.85, $p<0.0001$, respectively). This coronary microvascular dysfunction was independent of cholesterol levels. A repeat PET scan was carried out in 5 patients after 10.1±2.3 months of ERT; resting and hyperaemic MBF and CFR were unchanged after ERT (0.993±0.16 vs. 0.991±0.16 ml/g/min, p=ns; 1.56±0.29 vs. 1.71±0.3 ml/g/min, p=ns and 1.6±0.37 vs. 1.74±0.28, p=ns respectively).

Conclusions

The results of the present study demonstrate that patients with AFD have markedly abnormal coronary microvascular function. Our preliminary data suggest that ERT has

no effect on coronary microvascular dysfunction. Further work is necessary to determine whether treatment at an earlier stage in the course of the disease may improve coronary microvascular function in AFD patients.

Aims

Patients with AFD complain of angina despite angiographically normal coronary arteries. I have previously described a progressive deterioration in left ventricular systolic function and a previous study from this group has demonstrated myocardial scarring in patients with AFD cardiomyopathy[63, 121]. The aim of this study was to see if these clinical abnormalities may be caused by coronary microvascular dysfunction.

The aims of this study were to measure coronary flow reserve (CFR), an index of coronary microvascular function, in a consecutive cohort of patients with AFD using positron emission tomography (PET), and to determine the effect of ERT on microvascular abnormalities.

Methods

Patient and Controls

Ten non-smoking male patients with AFD (53.8 ± 10.9 years, range 43-82, cholesterol 5.5±1.3 mmol/l) referred to the Heart Hospital, London, UK were studied. The diagnosis of AFD was based on the identification of an α-Gal gene mutation and a low plasma α-Gal (mean 0.45 $\pm$ 0.35 nmol/hr/ml, range 0.04-0.99 nmol/hr/ml). Nine patients were identified through screening of patients presenting with unexplained left ventricular hypertrophy (LVH). One patient was referred with an established diagnosis of AFD (# 6), and 1 was identified through family screening; (# 10). At the time of diagnosis, none of these patients were receiving ERT.

All patients had ECG and 2-D/Doppler echocardiography performed using the previously described methods in chapter 2. Nine patients had symptoms and/or signs suggestive of myocardial ischaemia and underwent coronary angiography to exclude coronary artery disease.

Twenty four healthy non-smoking age matched males (52.0±8 years, cholesterol 4.5±0.6 mmol/l) with normal electrocardiograms (ECG), and no evidence of cardiac disease served as controls for the MBF and CFR data.

Enzyme Replacement Therapy

Five patients (Table 5.1) received Fabrazyme™ (Genzyme Corporation, Cambridge MA, USA) at a dose of 1 or 2 mg/kg every two weeks as part of a separate randomised study. Follow up PET scans were obtained 17.1±1.9 months after the baseline scan while patients were on treatment (mean 10.1±2.3 months on ERT). Plasma globotriaosylceramide (neutral glycosphingolipid) levels were measured using tandem mass spectrometry pre- and post ERT[122].

The study was approved by the Local Research Ethics Committees and all participants gave written informed consent. Radiation exposure was approved by the UK Administration of Radioactive Substances Advisory Committee (ARSAC).

Positron Emission Tomography (PET)

All patients and controls underwent PET scanning to measure MBF at rest and during adenosine-induced hyperaemia (140 μg/kg/min i.v.). Scanning was performed with an

ECAT 931-08/12 15-slice tomograph with a 10.5-cm axial field of view (CTI/Siemens, Knoxville, TN). Resting and hyperemic MBF were measured using oxygen-15 labeled water ($H_2{}^{15}O$) as previously reported[123]. Arterial blood pressure was recorded by automatic cuff sphygmomanometer at one-minute intervals and the ECG was monitored continuously throughout the procedure.

PET Data Analysis

All emission and transmission data were reconstructed using a Hanning filter with a cut-off frequency of 0.5 units of the reciprocal of the sampling interval of the projection data resulting in an image resolution of 8.4x8.3x6.6 mm full width at half maximum at the centre of the field of view. Myocardial and blood pool images were then generated directly from the dynamic $H_2{}^{15}O$ study as previously reported[124]. Regions of interest were drawn within the left atrium and ventricular myocardium on consecutive image planes. These were projected onto the dynamic $H_2{}^{15}O$ images to generate blood and tissue time activity curves. Arterial and tissue activity curves were fitted to a single tissue compartment tracer kinetic model to give values of MBF (ml/g/min)[124]. Coronary resistance (mmHg/ml/min/g) was calculated as the ratio of mean arterial pressure to MBF and CFR as the ratio of hyperaemic MBF to resting MBF.

Statistical Analysis

Comparisons between continuous variables were performed using the paired Student t-test (SPSS v19.0; SPSS, Inc., Chicago, Illinois, USA). Linear regression was performed to assess the relationship between cholesterol levels, LVM index, MBF and CFR. Data are reported as mean ± SD values. A $p<0.05$ was considered significant.

Results

Patient Characteristics

The clinical characteristics of the patients are shown in Table 5.1. Six (60%) patients complained of exertional chest pain. None of the nine patients had angiographically significant coronary artery disease. Three patients had rate responsive dual chamber permanent pacemakers: one for left ventricular outflow tract gradient reduction and two for heart block. None of the patients had a PR interval less than 120ms. Two patients had non-sustained ventricular tachycardia on Holter (# 2 and 3), and 2 were in atrial fibrillation (# 5 and 11). Seven years prior to the diagnosis of AFD, patient #1 had undergone an assessment of coronary sinus pH during dipyridamole infusion[125]. The peak change in coronary sinus pH during hyperaemia was -0.086 units, indicating severe myocardial ischaemia.

Serum cholesterol was higher in the patients compared to controls (mean difference 0.97 mmol/l, 95% CI = 0.3-1.6, p = 0.006). Patients also had higher HDL cholesterol levels compared to controls (1.7±0.5 mmol/l vs. 1.1±0.4 mmol/l, respectively, p = 0.002). There were no differences in LDL cholesterol (3.1±0.8 mmol/l vs. 3.7±1.3 mmol/l, patients vs. controls respectively, p = 0.2) and triglyceride levels (1.4±0.7 mmol/l vs. 1.8±1.6 mmol/l, patients vs. controls respectively, p = 0.5).

Two patients had acroparaesthesiae, 1 had hypohidrosis and 1 had renal dysfunction (creatinine clearance 52 ml/min). None of the patients were hypertensive and one was a type 2 diabetic. No angiokeratomata were noted in any of the patients. All patients

had a maximal left ventricular wall thickness ≥ 13mm (17 ± 4.0 mm, range 14-26 mm). Nine patients had concentric left ventricular hypertrophy (LVH) and 1 had asymmetric septal hypertrophy. At the time of study no patient had left ventricular outflow tract obstruction; the mean left ventricular end-systolic, end-diastolic and left atrial diameters were 33±0.9, 52±08 and 47 ±11 mm respectively. The mean LVM index was 234±81.3g/m^2 (range 141- 407 g/m^2).

Myocardial Blood Flow and Coronary Flow Reserve

Heart rate and mean arterial blood pressure were similar in controls and AFD patients at rest and during adenosine infusion. The rate pressure product (RPP= systolic blood pressure x heart rate) was similar in the controls and patients during all study conditions.

Table 5.1: Patient characteristics.

Patient	Age at study (years)	Chest pain	NYHA Class	Syncope	Palpitations	Max. LVWT (mm)	FS (%)	LVM index (g/m^2)	CFR
1	46	+	II	+	+	25	40	263	1.58
2*	58	-	I	+	+	26	26	407	2.60
3*	81	-	III	-	+	18	23	228	2.31
4	49	+	II	-	-	17	35	192	2.09
6*	55	+	II	-	+	15	36	222	2.24
7*	49	+	II	-	+	16	39	180	2.74
8	45	+	I	-	+	20	51	248	1.27
9*	54	-	I	-	-	24	42	314	2.27
10	43	-	I	-	-	15	33	93	1.28
12	58	+	II	+	+	20	27	407	1.83

Table 5.1 legend: NYHA=New York Heart Association, Max LVWT = maximal left ventricular wall thickness, FS = fractional shortening, %=percentage, CFR = coronary flow reserve, * = 5 patients with follow-up PET scans following Enzyme Replacement Therapy.

Resting and hyperaemic MBF and CFR were significantly reduced in AFD patients compared to controls (0.99±0.17 vs. 1.17±0.25 ml/g/min, p<0.05, 1.37±0.32 vs. 3.44±0.78 ml/g/min, p<0.0001 and 1.41±0.39 vs. 3.03±0.85, p<0.0001), respectively (Figure 5.1 and Table 5.1). This reduction in coronary microvascular function was independent of cholesterol and HDL cholestero1 levels. Resting coronary resistance was comparable in patients and controls (82.2±27.8 vs. 89.3±31.2 mmHg/ml/min/g p=ns respectively). The minimal resistance during hyperaemia was higher in AFD patients compared to controls (65.1±16.9 vs. 26.5±6.0 mmHg/ml/min/g p<0.0001). No correlation was found between LVM index and resting or hyperaemic MBF or CFR.

Response to ERT

In those patients that underwent a repeat PET scan there was a decrease in plasma globotriaosylceramide with ERT (mean change=5.3 µg/ml, 95% CI = 0.5, 10.1, p=0.04). Resting and hyperaemic MBF and CFR, before and after 10.1±2.3 months of ERT were similar (0.99±0.16 vs. 0.99±0.16 ml/g/min, p=ns, 1.56±0.29 vs. 1.71±0.3 ml/g/min, p=ns and 1.6±0.37 vs. 1.74±0.28, p=ns respectively, Figure 5.2). Resting coronary resistance was similar pre- and post- ERT (83.9±18.9 vs. 90.2±26.5 mmHg/ml/min/g, p=ns) respectively.

The minimal resistance during hyperaemia was unchanged following ERT (56.9±14.7 vs. 52.8±18.7 mmHg/ml/min/g, p=ns). There was no change in LVM index (mean change=0.4±37.8 g/m^2, ranging from -66.3 to +26.4, p=ns).

Figure 5.1: Myocardial Blood Flow at Rest and During Adenosine-Induced Hyperaemia in Patients with Anderson-Fabry Disease and Controls.

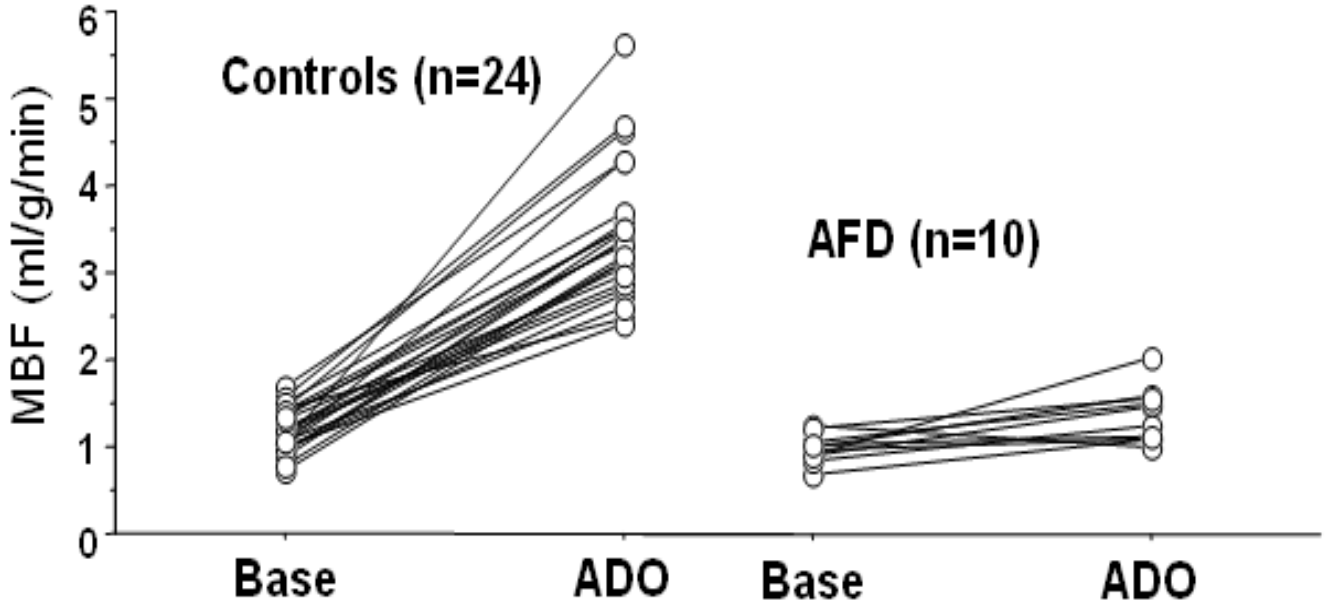


Figure 5.1 legend: MBF = Myocardial blood flow, Base = MBF at rest, ADO = MBF during adenosine.

Figure 5.2: Myocardial Blood Flow at Rest and During Adenosine-Induced Hyperaemia Pre- and Post Enzyme Replacement Therapy (Fabrazyme™).

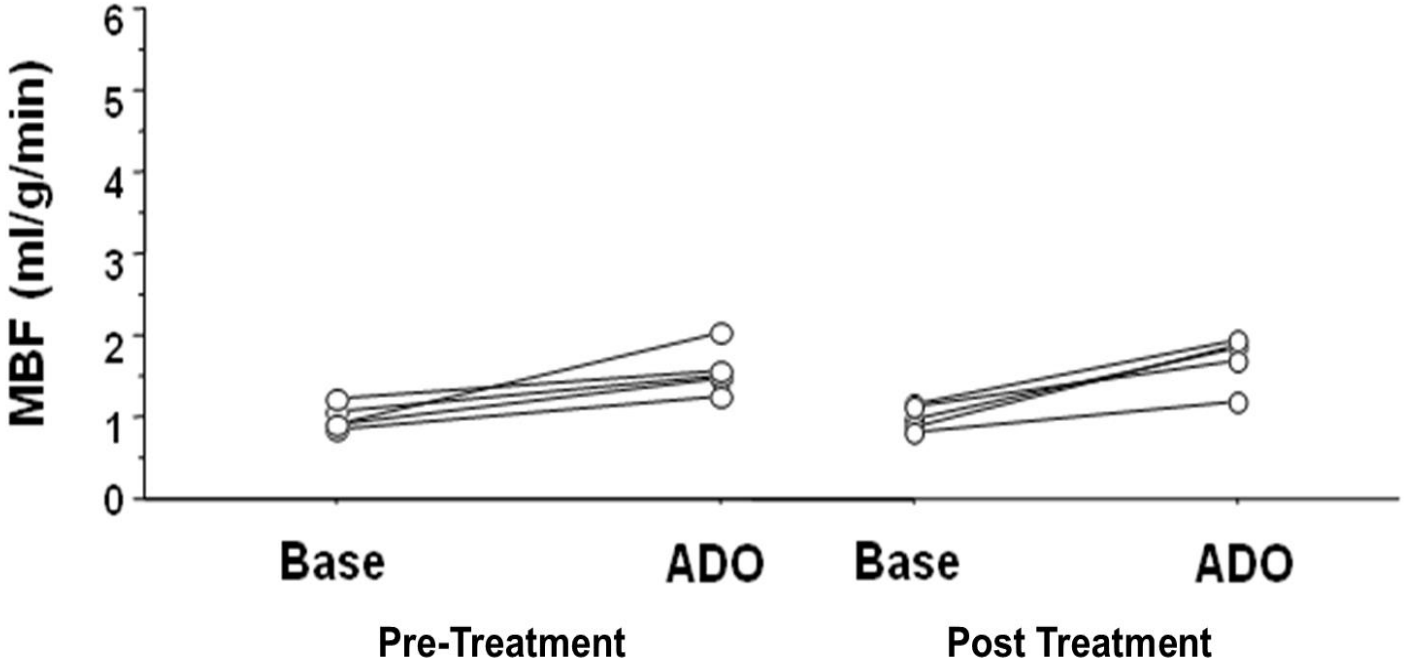


Figure 5.2 legend: MBF = Myocardial blood flow, Base = MBF at rest, ADO = MBF during adenosine.

There were no changes in the left ventricular cavity dimensions (mean change in end systolic cavity dimension=0.1±0.3mm, p=ns; mean change in end diastolic cavity dimension=0.1±0.2mm, p=ns).

There was a trend to a reduction in fractional shortening (mean fractional shortening pre-ERT=34.8±6.7%, mean post-ERT=31.4±6.3%, p=0.08). There was no change in the maximal left ventricular wall thickness (mean change=0.02±0.3mm, p=ns).

Discussion

This study shows that patients with AFD have a substantial reduction in hyperaemic MBF and CFR compared to normal controls. The significance of these findings for individual patients remains to be determined, but the severity of the microvascular dysfunction suggests that these abnormalities may have a substantial influence on the natural history of AFD.

Mechanisms of Microvascular Dysfunction

Symptoms and signs suggestive of myocardial ischaemia in the absence of coronary disease are frequent in patients with cardiomyopathies and in individuals with left ventricular hypertrophy secondary to pressure overload. Reductions in CFR similar to those seen in this study have been demonstrated in all these conditions, although different mechanisms are involved. In patients with hypertrophic cardiomyopathy, remodelling of the intramural arterioles is important[126,127], whereas in aortic stenosis, increased perivascular fibrosis and intramyocardial pressure and reduced diastolic filling are the dominant mechanisms for microvascular dysfunction[128, 129].

In AFD, a number of mechanisms may contribute to microvascular dysfunction. AFD cardiomyopathy is characterised by GB3 deposition in myocytes, conduction tissue, vascular endothelium and valvular tissue. This is accompanied by secondary changes such as myocyte hypertrophy and fibrosis[130] which cause raised coronary vascular resistance and increased myocardial oxygen demand. Although endothelial GB3 deposits may lead to microvascular dysfunction, a recent publication has shown that patients with AFD have *enhanced* nitric oxide independent endothelial function measured using forearm venous plethysmography[131]. It remains to be seen whether similar abnormalities are seen in the coronary microvasculature.

Response to ERT

In this study there was a significant reduction in the plasma levels of globotriaosylceramide with ERT, but no improvements in coronary microvascular function or LVM were seen. However, interpretation of this observation is limited by the small size of the cohort and by the fact that the cohort was considerably older, and had more severe cardiac disease compared to patients included in previous trials of ERT[73, 75, 132]. Along with an improvement in AFD symptoms these trials have demonstrated a reduction in LVM and an improvement in systolic function with ERT. In order to understand the impact of ERT on coronary microvascular function a larger study conducted over a longer period of time is required.

Conclusion

This study shows that microvascular dysfunction is important in the pathophysiology of AFD cardiomyopathy. Further studies are required to assess the impact of enzyme replacement therapy on microvascular function.

Chapter 6

Extracellular matrix turnover in Anderson-Fabry disease

Abstract

Background

Patients with AFD develop progressive left ventricular (LV) remodelling and heart failure. We hypothesized that altered extracellular matrix turnover (ECM) contributes to the pathophysiology of cardiac disease in AFD.

Methods and results

Twenty-nine (44.1±11.7 years, 15 male) consecutive patients with AFD and 21 normal controls (39.7±11.3 years, 10 male) had serum analysed for matrix metalloproteinase-9 (MMP-9), and tissue inhibitor of matrix metalloproteinase 1 and 2 (TIMP-1, TIMP-2). All patients underwent clinical assessment, echocardiography and Mainz Severity Score Index measurement (MSSI); a validated severity score in AFD.

MMP-9 levels were significantly higher in patients than controls (1003.8±337.8ng/ml vs. 576.7±276.3ng/ml respectively, $p < 0.001$). There were no differences in TIMP levels between patients and controls. There was a positive correlation between MMP-9 levels and MSSI ($r = 0.5$, $p=0.01$). There was a negative correlation between MMP-9 and fractional shortening (FS, $r = -0.5$, $p=0.01$). There was no correlation between LV mass or maximal LV wall thickness and MMP-9 levels. These relations were independent of age and gender using stepwise linear regression analysis.

Conclusions

Patients with AFD have abnormal ECM turnover compared to normal controls. The correlation between MMP-9 levels and FS suggests that altered ECM turnover is

important in cardiac remodelling. The association between MMP-9 and overall disease severity suggests that circulating levels of matrix metalloproteinases may provide a useful marker for assessing the response of patients with AFD to enzyme replacement treatment.

Aim

Previous chapters have described progressive left ventricular dysfunction, significant arrhythmia and significant exercise limitation.

Matrix metalloproteinases (MMPs) and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) are the key mediators of extracellular matrix (ECM) turnover, the balance between constitutive enzyme activity and inhibitor activity ultimately determining ECM composition for a variety of elements. While tissue composition is the key a variety of studies have suggested that the measurement of circulating MMPs and TIMPs are associated and provide a non-invasive assessment of ECM turnover[133-135]. For example circulating levels of TIMP-1 appear to correlate with the severity of structural left ventricular hypertrophy and related diastolic filling abnormalities in hypertensive heart disease[136, 137].

The aim of this study was to assess if patients with AFD have abnormal collagen turnover as compared to age matched normal healthy cohort and to assess its relationship to overall disease severity.

Methods

The study cohort comprised 29 consecutive patients (mean age 44.1 ± 11.7 years, 15 (60.0%) male and 14 (40.4%) female) with a genetically confirmed diagnosis of AFD and 21 age and gender matched healthy normal controls (mean age 39.7 ± 11.3 years, 10 (47.6%) male and 11 (52.4%) female). The latter were asymptomatic and had no clinical evidence of vascular, neoplastic, metabolic or inflammatory disease (assessed by

history, examination and routine laboratory analysis). All controls were normotensive (supine blood pressure <140/90 mmHg), and had a normal resting ECG. All were free of drug therapy.

The study was approved by the local research ethics committee and informed, written consent was obtained from all patients and controls.

Clinical Assessment

All patients underwent a complete clinical assessment with clinical history, examination, a standard 12 – lead electrocardiogram (ECG) and echocardiography as described previously in chapter 2.

Mainz Severity Score Index (MSSI)

In brief, the MSSI scoring system comprises four components: general, neurological, cardiovascular and renal (chapter 4, Table 4.1)[106]. Each component consists of a group of signs and symptoms associated with AFD, weighted according to their contribution to morbidity. For each sign and symptom, a score is assigned and summed to produce a total score for that component (chapter 4, Table 4.1). These individual component scores are then totalled to give the final MSSI score.

MMP-9, TIMP-1 and TIMP-2 Evaluation

Circulating concentrations of serum MMP-9, TIMP-1 and TIMP-2 were measured by an in house enzyme linked immunosorbent assay as described previously[135]. In brief, serum was stored in aliquots at -20ºC and batch analysed for MMP and TIMP.

Monoclonal primary and biotinylated secondary antihuman antibody for total MMP-9, TIMP-1 and TIMP-2 were tested against respective recombinant human MMP and TIMP (MAB911, BAF911, 911-MP; MAB970, BAF970, 970-TM; MAB9711, BAF971, 971-TM; R&D Systems Europe Ltd, Abingdon, UK) and against pooled human plasma to determine the optimal concentration of primary (2 μg/mL, 4 μg/mL and 2 μg/mL respectively) and secondary antibody (0.1 μg/mL, 0.1 μg/mL and 0.2 μg/mL respectively) sample dilution (1/100) and standard. The mean intra-assay coefficient of variation (CV) and mean inter-assay CV for the assays were <5% and <10% respectively.

Statistics

Statistical analysis was performed using SPSS v19.0. The χ^2 test was used to compare non-continuous variables. Normally distributed variables were compared using the 2-tailed independent sample Student t-test and non-normally distributed variables were compared using the Man Whitney U test. Correlations for normally distributed variables were expressed as a Pearson's correlation coefficient and non-normally distributed variables as a Spearman's rho coefficient. All values were expressed as means (standard deviation) for normally distributed variables and median (interquartile range) for non-normally distributed variables.

Stepwise linear regression was used to assess the relationship between circulating MMP and TIMP levels and markers of disease severity were adjusted for covariates. As females develop signs and symptoms of the disease at older ages, gender was entered into the regression model; age was entered into the regression model as there is a progressive accumulation of glycosphingolipid with advancing age.

Table 6.1: Baseline Characteristics in Males and Females

	Total	Female (14)	Male (15)	p-value
*TIMP-2 (ng/ml)	210 (190-250)	198.5 (188.8-265)	210 (190-240)	0.9
TIMP-1 (ng/ml)	1029.7 (299.3)	996.8 (276.2)	1060.3 (326.0)	0.6
MMP-9 (ng/ml)	1003.8 (337.8)	887.1 (307.9)	1112.7 (337.5)	0.07
Age (years)	44.1 (11.7)	45.7 (13.5)	42.5 (10.0)	0.5
$MSSI_{TOTAL}$	26.9 (14.7)	17.1 (10.4)	36.0 (12.1)	<0.001
$MSSI_{GENERAL}$	6.5 (4.2)	3.6 (2.1)	9.0 (4.1)	<0.001
$MSSI_{NEUROLOGICAL}$	5.6 (4.6)	4.2 (3.4)	6.9 (5.2)	0.1
$MSSI_{CARDIAC}$	8.7 (5.8)	5.2 (5.5)	11.9 (4.0)	0.001
$MSSI_{RENAL}$	5.5 (5.2)	3.1 (3.6)	7.7 (5.5)	0.01
PR interval (ms)	147.3 (30.1)	156.5 (35.4)	138.7 (22.0)	0.1
QRS Duration (ms)	93.2 (14.3)	82.9 (7.9)	102.9 (12.0)	<0.001
Max LV Wall Thickness (mm)	13.4 (4.3)	10.9 (1.8)	15.8 (4.5)	0.001
*RWT	0.5 (0.4-0.6)	0.4 (0.4-0.5)	0.6 (0.5-0.6)	0.01
LVed (mm)	47.4 (4.0)	45.9 (2.2)	48.7 (4.8)	0.06
LVes (mm)	29.0 (3.9)	27.4 (2.7)	30.4 (4.3)	0.04
FS (%)	38.7 (7.5)	40.1 (5.3)	37.3 (9.1)	0.3
*LV Mass Index (g/cm^2)	96.8 (91-145)	92.0 (82.6-95.8)	132.4 (101-183)	<0.001

Table 6.1 legend: TIMP = tissue inhibitor of matrix metalloproteinases; MMP = matrix metalloproteinase; MSSI = Mainz Severity Score Index; Max LV = maximal left ventricular; RWT = relative wall thickness; LVed = left ventricular end diastolic cavity dimension; LVes = left ventricular end systolic cavity dimension; FS = fractional shortening; * = non-normally distributed variables comparison made using Man Whitney U test, expressed as median (interquartile range), normally distributed variables expressed as mean (standard deviation).

Other covariates were entered into the model if there was a relationship between the covariate and MMP or TIMP with a p-value of < 0.05 on univariate analysis. Statistical significance was defined as $p < 0.05$.

Results

The clinical characteristics of the patients are shown in Table 6.1. MMP-9 levels were elevated in patients with AFD by a mean of 427.1 ng/ml (95% CI = 252.1, 602.2 ng/ml, $p < 0.001$, Figure 6.1). There were no differences between patients and controls in TIMP-1 and TIMP-2 levels.

Disease Severity and MMP-9

Table 6.1 describes each of the MSSI component scores stratified by gender. The mean MSSITOTAL score was higher in men compared to women (19.1, 95% CI = 10.7 to 27.5, Table 6.1).

Similarly, the mean MSSIGENERAL, MSSICARDIAC and MSSIRENAL but not MSSINEUROLOGICAL scores were higher in men compared to women (5.4, 95% CI = 2.7 to 7.6; 6.7, 95% CI = 2.9 to 10.4; 4.6, 95% CI = 1.0 – 8.2; 2.7, 95% CI = -0.6 to 6.1 respectively, table 6.1). There was a positive correlation between MMP-9 levels and MSSITOTAL, MSSIGENERAL and MSSINEUROLOGICAL, scores ($r = 0.5$, $p = 0.01$, Figure 6.2; $r = 0.4$, $p = 0.03$; $r = 0.4$, $p = 0.047$ respectively), but not with MSSICARDIAC or MSSIRENAL scores.

Figure 6.1: Difference in Matrix Metalloproteinase – 9 between Patients and Controls

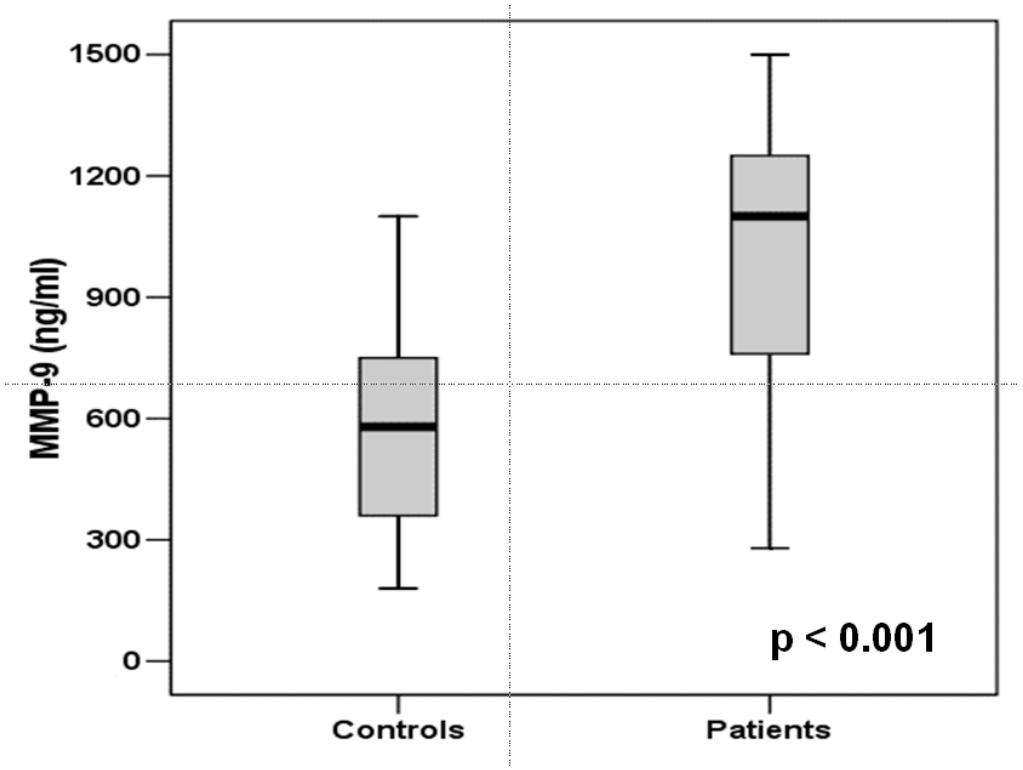


Figure 6.1 legend: Box and whiskers plot for controls and patients. Boxes represent median ± interquartile range (580ng/ml ± 920 ng/ml for controls and 1100 ng/ml ± 1220 ng/ml for patients, p< 0.001).

Figure 6.2: Correlation between Metalloproteinase – 9 and Disease Severity

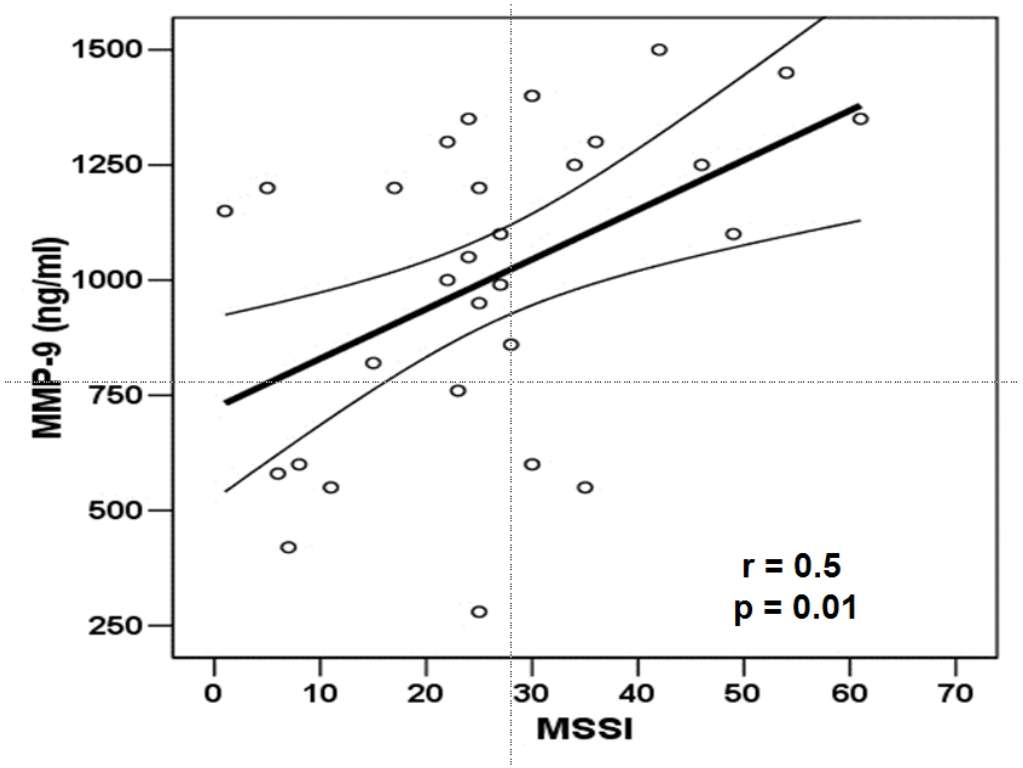


Figure 6.2 legend: Disease severity assessed as Mainz Severity Score Index ($MSSI_{TOTAL}$).

Figure 6.3: Correlation between Metalloproteinase – 9 and Fractional Shortening

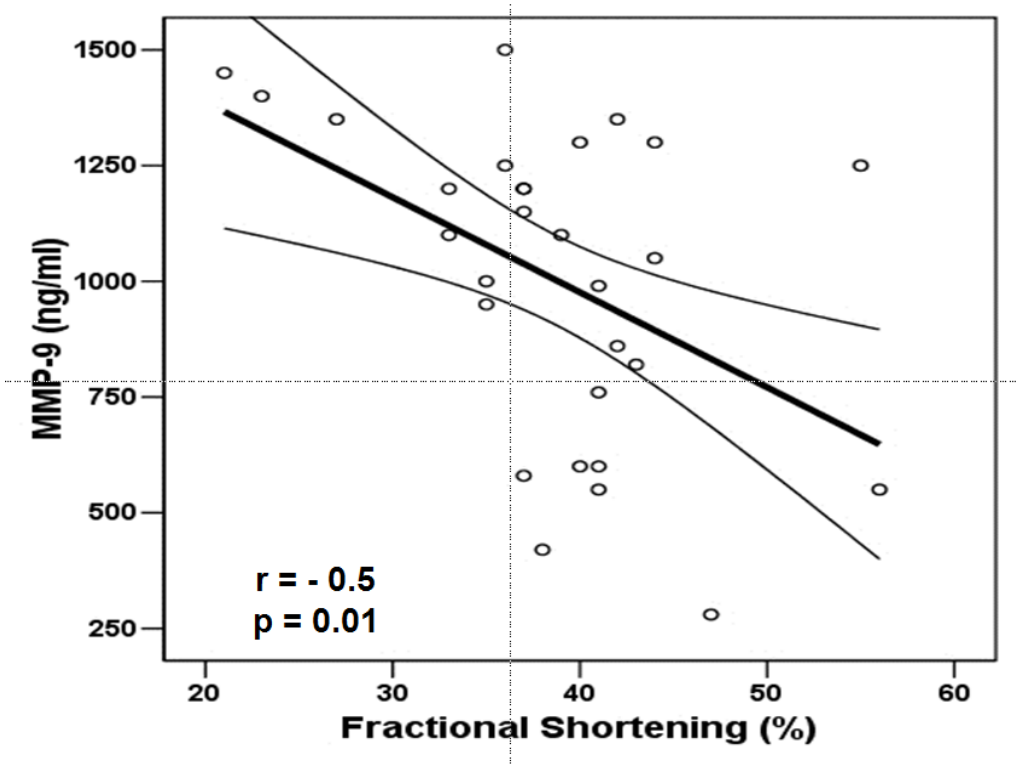


Figure 6.3 legend: Fractional shortening assessed on echocardiography and expressed as a percentage.

Cardiovascular Evaluation

Four of the 29 (13.8%) patients complained of angina, 5/29 (17.2%) were in NYHA class 2 or greater, 8/29 (27.6%) complained of palpitations and none had experienced pre-syncope/syncope. There were no gender differences in the frequency of cardiac symptoms.

All patients were in sinus rhythm. The mean PR interval was 147.3 ± 30.1 ms (100 – 223 ms) with a mean QRS duration of 93.2 ± 14.3 ms (72 – 132 ms). Men had a higher QRS duration when compared to women (mean difference = 19.9 ms, 95% CI = 12.1ms to 27.8ms, $p < 0.001$, Table 6.1).

Seventeen (58.6%, 12 males and 5 females) patients had evidence of cardiac remodelling. Eight (27.6%, seven males) had evidence of concentric hypertrophy, 9 (31.0%, 5 males) had concentric remodelling and 12 (41.4%, 3 males) had normal LV geometry.

The mean maximum LV wall thickness was 13.4 ± 4.3 mm (8 - 26mm), with a mean RWT of 0.5 ± 0.1 (0.3 - 1.0) and a mean LVMI of 121.3 ± 48.4 gm/m^2. Men had higher maximal LV wall thickness (mean of 4.9mm, 95% CI = 2.3mm to 7.6mm); higher RWT (mean of 0.1, 95% CI = 0.03 to 0.2); and higher LVMI (mean 56.1 gm/m^2, 95% CI = 25.8 to 86.4 gm/m^2) when compared to women (Table 6.1). Men had a higher LVes, a trend to higher LVed and no difference in the FS when compared to women (Table 6.1).

There was a negative correlation between MMP-9 levels and FS ($r = -0.4$, $p = 0.02$, Figure 6.3). There was a trend to a positive correlation between MMP-9 levels and QRS

duration (r = 0.4, p = 0.047) and RWT (r = 0.4, p = 0.06). MMP-9 levels did not correlate with LVMI or left ventricular cavity dimensions.

Stepwise linear regression demonstrated a relation between MMP-9 levels and MSSI score and FS that was independent of age and gender (β = 11.0, 95% CI = 3.9 to 18.7, p = 0.004; β = -20.2, 95% CI = -6.4 to -33.9, p = 0.006).

Discussion

This study demonstrates that patients with AFD have elevated levels of circulating MMP-9 when compared to normal controls. MMP-9 levels correlated with clinical markers of disease severity, suggesting that abnormal ECM turnover plays an important role in the pathogenesis of AFD.

MMP in Heart Disease

The ECM is composed of a wide range of elements including collagen, elastin, specialised proteins (fibrillin and fibronectin) and proteoglycans. It has an important role in the maintenance of the structural integrity and function of organs and influences both intracellular signalling and cell-to-cell interactions. Although the ECM is ubiquitous, its composition can vary from organ to organ[138].

There are more than twenty different MMP's, each acting on a number of ECM substrates[138, 139]. MMP-9, also known as gelatinase, is a 92-kD protein secreted by myocytes, fibroblasts, smooth muscle cells and neutrophils; its function is to degrade a number of interstitial proteins including basement membrane components such as laminin and fibronectin[138].

Increased MMP expression occurs in several disease states including tumour angiogenesis and metastasis, rheumatoid arthritis and vascular disease[140, 141]. It has been shown recently that circulating levels of MMP-9 predict disease severity and prognosis in stable and unstable coronary artery disease, peripheral arterial disease and hypertension[134, 142, 143]. Myocardial and blood levels of MMP-9 are elevated in patients with ischaemic and non-ischaemic left ventricular dilatation[144, 145]. In the low risk population studied in The Framingham Heart Study blood levels of MMP-9 were associated with increased left ventricular cavity dimensions and mass index[146]. TIMP-1 activity is increased in hypertension and diabetes and is associated with myocardial fibrosis and diastolic dysfunction [137]; TIMP-2 has been linked with angiogenesis[147].

Significance of ECM Turnover in AFD

Although the genetic and biochemical basis of AFD is well described, the pathophysiology of cardiac disease in AFD is inadequately understood. Histologically, AFD cardiomyopathy is characterised by myocyte hypertrophy and vacuolation[97]. However, substrate accumulation accounts for only 1% of the increased LV mass seen in AFD cardiomyopathy[62].

The findings in this study support recent data from cardiac magnetic resonance imaging suggesting that myocardial fibrosis may be a key determinant of cardiac function in AFD[63]. The main impact of elevated MMP-9 levels is to increase collagen type I / III ratio[139]. Collagen type I is not a major substrate for MMP-9, and hence raised levels of MMP-9 may reflect the breakdown of components other than collagen type I[139].

Although TIMP-1 directly inhibits the degradation of collagen type I in the ECM, there were no differences in circulating levels between patients and controls. It is possible that elevated MMP-9 levels may directly increase collagen gene expression in the absence of elevated TIMP-1 levels[148]. Moreover, it is likely that the balance between TIMP and MMP activities is more important than abnormalities in either factor in isolation.

Monitoring of Disease Activity

The recent introduction of enzyme replacement therapy for the treatment of AFD represents a major advance in the treatment of lysosomal diseases[75]. A new challenge for clinicians treating such diseases is the monitoring of response to therapy. In some diseases there are highly sensitive circulating markers (e.g. chitotriosidase in Gaucher disease) that can be used; in AFD plasma and urine levels of Gb3 have been used, but recent evidence suggests that this may not be the most appropriate marker for monitoring response to treatment[122]. The association between MMP-9 and overall disease severity suggest that ECM turnover may be a useful alternative surrogate for the response to enzyme replacement treatment

Conclusion

Patients with AFD have abnormal ECM turnover; the relation between MMP-9 levels and disease severity suggests that altered ECM remodelling is central to the pathogenesis of AFD related complications.

Chapter 7

Summary of findings and current literature

Summary of findings and current literature

The aim of this thesis was to perform the first detailed characterisation of cardiac manifestations of AFD and to explore their relation to outcomes. Many of the findings were completely novel and have been confirmed in subsequent studies from our own group and other investigators.

Arrhythmia

In *chapter two*, I demonstrated that there is an increase in prevalence of both supraventricular and ventricular arrhythmia with age and that approximately 5% of patients required a permanent pacemaker at follow up. These findings suggested that cardiac arrhythmia may be a significant contributor to long-term morbidity and mortality in AFD. The high contribution of arrhythmia to symptoms has been confirmed in subsequent studies reporting palpitations or documented arrhythmia in 19.3% of the population in the international Fabry outcome survey not on ERT and 34.5% of the population on ERT[119]. Older patients with AFD are more likely to develop bundle branch block and atrioventricular conduction/sinus node disease[149] and in the FOS registry 3% of these patients required antibradycardia pacing[119]. Additionally most males have a resting bradycardia and chronotropic incompetence (also described in chapter 4)[150] and male children have reduced heart rate variability[151].

Work from our own cohort has shown that PR interval and QRS duration increase with age and are independent predictors of future anti-bradycardia pacing (hazard ratio HR 1.03, 95% CI 1.004–1.060, p = 0.02; HR 1.05, 95% CI 1.02–1.09, p < 0.001 respectively)[152]. In this study the annual implant rate for any cardiac device (anti-bradycardia or high

energy defibrillator device) was 2.1% (95% CI = 1.2-3.1) with a 5 year cumulative incidence of 12% (95% CI = 6.1-17.9%)[152].

Although the high prevalence of tachyarrhythmia has been documented in chapter 2 and other studies, its impact on the natural history of the disease is still uncertain. In rare instances it may be the first presentation of AFD cardiomyopathy[153]. Sudden cardiac death is rare in AFD, however it is likely from published literature that bradycardia as well as ventricular tachycardia would contribute[95, 154]. Weidemann *et* al followed 40 patients with advanced AFD on ERT and demonstrated a sudden cardiac death risk of 15% (6/40, total of 7 deaths in cohort)[155]. All of these patients had documented non-sustained ventricular tachycardia and myocardial fibrosis on cardiac MRI scanning[155]. Further research is needed to identify those at greatest risk and institute appropriate preventative therapies for the prevention of sudden cardiac death[156].

Histological examination of the conduction tissue demonstrates that the greater the involvement of the conduction tissue with GB3 deposition the greater the accelerated conduction with prolonged refractoriness and the greater the electrical instability[76, 157].

With the high prevalence of stroke in patients with AFD it is likely that AF contributes to this, but there are no data regarding thromboprophylaxis for patients with AFD cardiomyopathy and documented AF[44]. The pathophysiology of cerebrovascular disease is not completely understood, but is likely to be multifactorial including microvascular disease, increased thrombotic potential, arteriopathy and

thromboembolic[49-51]. Although risk stratification algorithms such as CHA_2DS_2-VASc score have been validated in the general population, it is unlikely that this would be applicable to the AFD population as the age of onset of atrial arrhythmia is much younger than in the general population[91]. It would seem intuitive that they should be considered for formal anticoagulation, particularly if there are high risk factors such as a mutation in the Factor V Leiden gene[90].

Left ventricular systolic function

Chapter 3 described the progressive deterioration in LV systolic function in a small cohort of patients with severe AFD cardiomyopathy. This change occurred without change in LV mass index and suggested that systolic function may be a useful marker for the assessment of disease progression and response to treatment. The relationship with FS and disease progression is likely to be related to inferior posterior myocardial scar that is seen on histology and CMR and is a limitation of the study[76, 158].

The most common finding in AFD cardiomyopathy is that of LV hypertrophy and is associated with cardiac symptoms[119]. The prevalence of LV hypertrophy increases with age, occurring earlier in males than in females and inversely associated with renal function[119]. Early stages of AFD cardiomyopathy are characterised by concentric LV remodelling which progresses to LV hypertrophy with time, with asymmetric septal hypertrophy seen in 5% of cases[119]. Additionally, right ventricular hypertrophy is also seen, usually in association with LV hypertrophy[159]. Right ventricular hypertrophy is seen in equal frequencies in males and females[159]. It becomes increasingly recognised with advancing age. The impact of right ventricular hypertrophy on right ventricular

function is contradictory in the literature[159, 160] and its impact on prognosis remains to be determined.

While echocardiography remains the mainstay for diagnosis of LV hypertrophy, there have been surprising few studies that have examining the change in systolic function in prospective cohorts. Tissue Doppler imaging appears to be a sensitive marker for early AFD cardiomyopathy with longitudinal velocities being depressed in the lateral and septal aspects of the mitral valve annulus prior to overt LV hypertrophy[116]. These tissue Doppler indices (in-vivo) correlate with resting and active tension in cardiomyocytes (in-vitro) but not with cardiomyocyte area, % storage of GB3 or % fibrosis[161]. These findings suggest that myofilament degradation and dysfunction contribute to systolic impairment[161]. Importantly, tissue Doppler indices appear to improve with enzyme replacement therapy, unless there is advanced AFD cardiomyopathy present[74, 162]. A recent study has suggested that a binary appearance of the left ventricular endocardial border on 2-dimensional echocardiography is a highly sensitive and specific discriminator of AFD from hypertrophic cardiomyopathy, however our group have not been able to reproduce this finding[163, 164].

Exercise limitation

In *chapter 4* I demonstrated that exercise limitation is common in males and in a substantial minority of females. The degree of impairment was not only related to severity of cardiac involvement, but also to overall disease severity as expressed by the MSSI score. This relationship was not influenced by the degree of proteinuria or GFR.

Additionally, males tended to have a degree of chronotropic incompetence which may have contributed to their exercise limitation.

Exercise limitation assessed with cardiopulmonary exercise testing has subsequently been demonstrated in several studies. Bierer *et al.* demonstrate a higher proportion of patients with abnormal peak exercise oxygen consumption as compared to controls[165]. The main finding from this study was a significant drop in diastolic blood pressure in a significant proportion of patients with exercise compared to controls[165]. This was not apparent in my study. In a follow up study they demonstrated an improvement in exercise capacity with ERT[166]. The drop in diastolic blood pressure seen in their original study was not replicated in the follow up study nor was there clear evidence for change in diastolic blood pressure response to exercise with ERT, particularly in patients included in both studies[166]. Lobo *et al.* demonstrate similar exercise limitation with cardiopulmonary exercise testing, but their follow up cohort failed to show an improvement in exercise capacity with 1 year of ERT[150]. These conflicting data question the utility of peak exercise oxygen consumption as a way of monitoring response to treatment.

An interesting study by Spinelli *et al.* where patients with AFD underwent radionuclide angiography during rest and exercise along with resting echocardiography and cardiac MRI scanning and compared to controls[167]. The study demonstrates that the majority of patients compared to controls had impaired exercise capacity. This impaired exercise capacity was associated with a failure to augment stroke volume with exercise and significant proportion having a reduction in stroke volume with exercise. This study

suggests that the predominant mechanism for exercise intolerance is cardiac dysfunction as opposed to pulmonary, musculoskeletal or hypohidrosis.

Coronary microvascular dysfunction

Chapter 5 demonstrates that patients with AFD cardiomyopathy have coronary microvascular dysfunction when compared to normal controls and that this does not improve with enzyme replacement therapy, at least in the short-medium term. These findings have been confirmed by other studies[168, 169]. Up to 20% of patients experience anginal symptoms[119] despite obstructive coronary artery disease being present in a minority of patients[10]. Volumetric intravascular ultrasound analysis of the coronary tree in patients with AFD and chest pain showed that patients have diffuse involvement of their coronary arteries with plaques that are less echogenic[170]. These findings are probably caused by Gb3 accumulation and disease-specific trophic influences rather than accelerated atherosclerosis[171]. Histologically, there is a diffuse storage arteriopathy affecting all parts of the arterial wall resulting in intimal thickening and vacuolation as well as medial hypertrophy[76]. Additionally, there is significant calcification associated with the arteriopathy, as in classical atherosclerotic disease; however seems to be present in the media as opposed to the intima[170]. Chimenti *et al*. have suggested that luminal narrowing of intramural arteries (due to GB3 deposition and hypertrophy in smooth muscle cells and endothelium) correlates with coronary flow and histological myocardial fibrosis[172]; suggesting that small vessel disease contributes to angina symptoms, myocardial fibrosis and potentially progressive heart failure in AFD cardiomyopathy[172].

Extracellular matrix turnover

Chapter 6 demonstrates that patients with AFD have abnormal extracellular matrix (ECM) turnover; the relation between MMP-9 levels and disease severity, as measured with the Mainz severity score index (MSSI), suggests that altered ECM remodelling is central to the pathogenesis of AFD related complications. My study demonstrates that circulating MMP-9 is elevated in patients with AFD. In patients with hypertrophic cardiomyopathy (HCM) caused by sarcomeric gene mutations, circulating MMP-2 levels and not MMP-9 levels are positively associated with left ventricular end systolic diameter, left atrial dimension and left ventricular systolic function[173]. The same study also suggests that MMP-2 in HCM was associated with worse symptoms, higher circulating B type natriuretic peptide (BNP), and worse prognosis[173].

A recent study of N-terminal proBNP (NT-proBNP) in patients with AFD demonstrated that concentrations are increased in patients with AFD and correlate with non-invasive markers of diastolic dysfunction[174]. Increased NT-proBNP levels were present in patients without echocardiographic evidence of LV hypertrophy[174]. These findings suggest that measurement of NT-proBNP levels might assist in decisions on the timing of ERT[174].

Cardiac magnetic resonance (CMR) imaging studies suggest that 50% of males and females with AFD have late gadolinium enhancement in posterior LV wall segments[63, 175]. This late enhancement seen in AFD cardiomyopathy correlate with myocardial collagen deposition when comparing histologically with pre-mortem CMR scans (Figure 1.5)[158]. Importantly this macroscopic change in the extracellular matrix is associated

with a lack of regression of left ventricular hypertrophy or improvement in segmental myocardial function with ERT[162, 176].

Although a deficiency in A-Gal activity results in GB3 accumulation in numerous cell types, it is clear that this is only the start of a cascade of cellular signalling activation that lead to hypertrophy, apoptosis, necrosis and fibrosis. The mechanisms underlying this cascade remain largely unknown. Recently it has been suggested that a circulating growth promoting factor perhaps lyso-Gb3, may have a causative role in the development of LVH in the hearts of patients with AFD[171, 177]. Another mechanism suggested for these cardiac changes was reported by Shen *et al*. They reported that excess intracellular GB3 induces oxidative stress and up regulation and expression of cellular adhesion molecules in vascular endothelial cells of patients, resulting in dysfunction of the coronary microvascular bed[178]. It has also been reported that GB3 accumulation alters mitochondrial energy metabolism. Alterations in mitochondrial metabolism in the LV wall of patients with sarcomeric HCM can be replicated in skin fibroblasts from patients with AFD disease, suggesting that GB3 deposits may cause disturbances in respiratory-chain activity, leading to reduced levels of creatinine phosphate, ADP, and ATP [179-181].

Screening for AFD in defined populations

I discussed new born screening for AFD in the introduction. These specific studies have identified a higher than expected incidence of AFD. These reported incidences are likely to still be an underestimation of the true incidence due to a failure to detect females and misdiagnosed cases. New born screening for several other conditions

already exists and the addition of AFD to this would not pose a technical challenge. However, children lost to follow up, false positive rates, missed female cases and the lack of prospective longitudinal data to demonstrate the efficacy of this strategy[28-30, 32-34] result in an ethical dilemma of whether there should be wide spread use of this strategy.

An alternative strategy is to target high risk populations and establish the prevalence of AFD in these populations (case finding studies) and then to screen the family of the identified individuals. These populations include patients on dialysis, with unexplained LVH and patients with cryptogenic stroke. A systematic review of the literature by Linthorst *et al* in 2014 identified 20 studies (10 of which looked at males and females) that looked at screening of high risk populations. The screening method was the identification of individual cases using A-Gal activity and then confirmatory genetic testing. The overall prevalence for AFD in men on dialysis was 0.33% (95% CI 0.20% to 0.47%) and 0.10% (95% CI 0% to 0.19%) in females. The prevalence of AFD in males with renal transplants was 0.33% (95% CI 0.07% to 0.69%) and 0% in females. Methodological differences in the selection of the study population and screening techniques in the studies looking at patients with unexplained LVH hampered the calculation of the overall prevalence which ranged from 0.9% to 3.9% in men and 1.1% to 11.8% in women. In premature strokes (n=2 studies), overall AFD prevalence was 4.2% (95% CI 2.4% to 6.0%) in men and 2.1% (95% CI 0.5% to 3.7%) in women[182]. Interestingly, most of the studies looking at screening females for AFD used A-Gal activity, even though it is known that this method fails to diagnose up to a third of patients and hence the prevalence in females is likely to be an under estimate[182].

Disease targeted therapies in AFD

Since I completed the work in thesis, the impact of ERT has been evaluated in several observational studies. Several have demonstrated progression of cardiac disease (especially in those with established cardiac involvement) despite ERT[76, 155, 183]. In the latest review from the Cochrane database of ERT, it concluded that 'there is no robust evidence for ERT in AFD'[184]. This may be in part to potential problems with the poor uptake of enzyme in cardiac and renal cells but is almost certainly also affected by the relatively late stage of disease at which ERT is commenced[185, 186]. However, while robust evidence showing that ERT prevents hard endpoints is still lacking, treatment is not futile as symptoms such as neuropathic pain, hypohidrosis/anhidrosis improve and there is a reduction in the rate of progression of renal disease all resulting in an improved quality of life[72, 73, 75, 155, 187-190].

The two available ERT agents are produced in different ways as described in the introduction and infused at different doses. Agalsidase alfa is infused at 0.2 mg/kg biweekly and agalsidase beta is infused at 1.0 mg/kg biweekly. There are limited data available for head to head efficacy comparison. Vedder *et al* showed no effect when both products were dosed at 0.2 mg/kg biweekly[191], however Beer *et al*, Imbriaco *et al* and Weidemann *et al* reported that left ventricular mass and measures of myocardial function were improved with agalsidase beta (1.0mg/kg biweekly) treatment[74, 162, 176, 192]. Hughes *et al* and Baehner *et al* reported improved left ventricular mass in patients treated with agalsidase alfa[193, 194].

A recent systematic review (including 31 studies) of the efficacy of ERT based on the stage of disease progression demonstrates that patients with a GFR>60 ml/min/1.73 m^2, the decline of renal function was similar for treated and untreated patients (historical controls), however males with a GFR<60 ml/min/1.73 m^2 had a slower rate of decline in renal function, possibly attributable to anti- proteinuric therapy. Regardless of baseline LVH, LV mass remained stable or increased, albeit at a slower rate compared to historical controls when treated with ERT. In females with LVH, LV mass decreased and in those without LVH, LV mass remained stable on ERT compared to historical controls. There was no evidence to support the use of ERT to treat white matter lesions in AFD in this systematic review[195]. This study highlights the difficulties in assessing the effectiveness of ERT in this chronic disease, there appears to be a differential effect of ERT in different organ systems as well as differing effects at different disease stages. The true long term efficacy of ERT in this chronic disease remains uncertain.

Small molecule 'chaperone therapy' either alone or in combination with ERT may also offer hope to patients with this disease[196]. These molecules are inhibitors of the enzyme and can increase the catalytic activity of mutated forms of the enzyme, however, approximately 45% of patients have nonsense mutations in the A-Gal gene resulting in very low levels of the enzyme. Future techniques to identify patients suitable for this therapy will assist in deciding who will benefit from this form of therapy[197].

Several other therapies are under investigation. *Substrate reduction therapy* is accomplished by blocking specific enzymatic steps in the biosynthesis of the accumulating substance. This form of therapy has been shown to help some patients with Gaucher Disease and has seen interest in the AFD community[198]. The effect of an inhibitor of glucocerebroside biosynthesis was examined in Fabry knockout mice. There was a 50% reduction in Gb3 in kidneys, liver and heart following 8 weeks of intra-peritoneal therapy with the inhibitor[199]. The precise mechanism of Gb3 clearance is unclear, but deserves further investigation.

The availability of the A-Gal knockout mice has made it possible to carry out critical experiments in regards to eventual *gene therapy* in patients with AFD[200]. A single injection with a recombinant adeno-associated viral vector containing modified chicken α-actin promoter resulted in normalisation of Gb3 levels in the liver and spleen, with an 85% reduction in the heart and 66% reduction in the lungs at 6 months. The kidney demonstrated an 82% reduction at 2 months, but had returned to 60% of the pre-treatment levels at 6 months[201, 202]. These are promising results that need further investigation, however gene therapy in this and many other metabolic conditions have been delayed due to the occurrence of insertional mutagenesis causing leukaemia in human recipients[203]. There is hope that these oncogenic hazards will be eliminated by using self-inactivating lentival vectors in stem cell derived erythroid cells[204].

Limitations of this thesis

Work subsequent to mine has demonstrated that there are techniques to identify AFD related cardiac disease early using advanced echocardiographic techniques such as

tissue Doppler and strain rate imaging[74, 155, 162]. These were not in wide usage at the time of my studies and although FS was seen to decline in patients with advanced AFD, this is likely a reflection of progressive replacement fibrosis that occurs with disease progression[76]. Additional imaging modalities such as cardiac MRI scanning, which has now been established as a method of differentiating underlying aetiologies of unexplained LVH would have added to this body of work[158]. Additionally, subsequent work has suggested that other biomarkers such as brain naturetic peptide may be useful in identifying patients with early cardiac involvement with AFD cardiomyopathy and exploring this and other biomarkers would have added to this body of work[174].

Conclusions

Together the chapters in my thesis demonstrate that progression of AFD cardiomyopathy is associated with cardiac systolic dysfunction and progressive arrhythmic disturbance. These changes are associated with exercise limitation. The underlying mechanisms are not clear, but it seems likely that coronary microvascular dysfunction and changes in the extracellular matrix play a role in the pathogenesis. There is a suggestion that early initiation of ERT, prior to cardiac fibrosis, may prevent progression of AFD cardiomyopathy, this however remains to be proven.

References

1. Desnick RJ, Ioannou YA, Eng CM. A-galactosidase a deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill; 2001:3733-3774.
2. Vellodi A. Lysosomal storage disorders. *Br J Haematol*. 2005;128:413-431
3. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *Jama.* 1999;281:249-254
4. Poorthuis BJ, Wevers RA, Kleijer WJ, Groener JE, de Jong JG, van Weely S, Niezen-Koning KE, van Diggelen OP. The frequency of lysosomal storage diseases in the netherlands. *Human Genetics*. 1999;105:151-156
5. de Duve C. Exploring cells with a centrifuge. *Science*. 1975;189:186-194
6. de Duve C. From cytases to lysosomes. *Fed Proc*. 1964;23:1045-1049
7. de Duve C. Lysosomes revisited. *Eur J Biochem*. 1983;137:391-397
8. Hers HG. Alpha-glucosidase deficiency in generalized glycogenstorage disease (pompe's disease). *Biochem J*. 1963;86:11-16
9. Baudhuin P, Hers HG, Loeb H. An electron microscopic and biochemical study of type ii glycogenosis. *Lab Invest*. 1964;13:1139-1152
10. MacDermot KD, Holmes A, Miners AH. Anderson-fabry disease: Clinical manifestations and impact of disease in a cohort of 98 hemizygous males. *Journal of Medical Genetics*. 2001;38:750-760
11. Anderson W. A case of angiokeratoma. *Br J Dermat*. 1898;1:113-117
12. Fabry J. Ein beitrag zur kenntnis der purpura haemorrhagic a nodularis (purpura papulosa haemorrhagic a hebrae). *Arch Dermatol Syph*. 1898;43:187-200
13. Linhart A, Elliott PM. The heart in anderson-fabry disease and other lysosomal storage disorders. *Heart*. 2007;93:528-535

14. Steiner L, Voerner H. Angiokeratoma miliare, eineideio-pathische gefaesserkrankung. *Deutsch Arch Klin Med*. 1909;96:105

15. Weicksel J. Angiomatosis bezw, angiokeratoma universalis. *Deutsch Med Woschr*. 1925;51:898 - 900

16. Ruiter M, Pompen AWM. Angiokeratoma corporis diffusum (universale) mit kardiovasorenale m symptomenkomplex bei 3 brudern. *Arch Derm Syph*. 1939;179:169

17. Pompen AW, Ruiter M, Wyers HJ. Angiokeratoma corporis diffusum (universale) fabry, as a sign of an unknown internal disease; two autopsy reports. *Acta Med Scand*. 1947;128:234-255

18. Scriba K. Zur pathogenes. Des angiokeratom a corporis diffusum fabry mit cardio-vasorenale m symptomenkomplex verhandlg. *Deutsch Path Gesellsch*. 1950;34:221

19. Colley JR, Miller DL, Hutt MS, Wallace HJ, De Wardener HE. The renal lesion in angiokeratoma corporis diffusum. *Br Med J*. 1958;1:1266-1268

20. Ruiter M. [angiokeratoma corporis diffusum syndrome and its cutaneous manifestations; review and personal experience in the last ten years]. *Hautarzt*. 1958;9:15-19

21. De Groot WP. Genetic aspects of the thesaurismosis lipoidica hereditaria ruiter-pompen-wyers (angiokeratoma corporis diffusum fabry). *Dermatologica*. 1964;129:281-282

22. Hashimoto K, Gross BG, Lever WF. Angiokeratoma corporis diffusum (fabry). Histochemical and electron microscopic studies of the skin. *J Invest Dermatol*. 1965;44:119-128

23. Dempsey H, Hartley MW, Carroll J, Balint J, Miller RE, Frommeyer WB, Jr. Fabry's disease (angiokeratoma corporis diffusum): Case report on a rare disease. *Ann Intern Med*. 1965;63:1059-1068

24. Opitz JM, Stiles FC, Wise D, Race RR, Sanger R, Von Gemmingen GR, Kierland RR, Cross EG, De Groot WP. The genetics of angiokeratoma corporis diffusum (fabry's disease) and its linkage relations with the xg locus. *Am J Hum Genet*. 1965;17:325-342

25. Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in fabry's disease. Ceramidetrihexosidase deficiency. *N Engl J Med*. 1967;276:1163-1167

26. Desnick RJ, Simmons RL, Allen KY, Woods JE, Anderson CF, Najarian JS, Krivit W. Correction of enzymatic deficiencies by renal transplantation: Fabry's disease. *Surgery*. 1972;72:203-211

27. Brady RO, Tallman JF, Johnson WG, Gal AE, Leahy WR, Quirk JM, Dekaban AS. Replacement therapy for inherited enzyme deficiency. Use of purified ceramidetrihexosidase in fabry's disease. *N Engl J Med*. 1973;289:9-14

28. Inoue T, Hattori K, Ihara K, Ishii A, Nakamura K, Hirose S. Newborn screening for fabry disease in japan: Prevalence and genotypes of fabry disease in a pilot study. *J Hum Genet*. 2013;58:548-552

29. Hopkins PV, Campbell C, Klug T, Rogers S, Raburn-Miller J, Kiesling J. Lysosomal storage disorder screening implementation: Findings from the first six months of full population pilot testing in missouri. *J Pediatr*. 2015;166:172-177

30. Ozkara HA, Topcu M. Sphingolipidoses in turkey. *Brain Dev*. 2004;26:363-366

31. Pinto R, Caseiro C, Lemos M, Lopes L, Fontes A, Ribeiro H, Pinto E, Silva E, Rocha S, Marcao A, Ribeiro I, Lacerda L, Ribeiro G, Amaral O, Sa Miranda MC. Prevalence of lysosomal storage diseases in portugal. *Eur J Hum Genet*. 2004;12:87-92

32. Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ. High incidence of later-onset fabry disease revealed by newborn screening. *Am J Hum Genet*. 2006;79:31-40

33. Hwu WL, Chien YH, Lee NC, Chiang SC, Dobrovolny R, Huang AC, Yeh HY, Chao MC, Lin SJ, Kitagawa T, Desnick RJ, Hsu LW. Newborn screening for fabry disease in taiwan reveals a high incidence of the later-onset gla mutation c.936+919g>a (ivs4+919g>a). *Hum Mutat*. 2009;30:1397-1405

34. Matern D, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P, Tortorelli S. Newborn screening for lysosomal storage disorders. *Semin Perinatol*. 2015;39:206-216

35. MacDermot KD, Holmes A, Miners AH. Anderson-fabry disease: Clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. *J Med Genet*. 2001;38:769-775

36. Germain DP. Fabry disease. *Orphanet J Rare Dis*. 2010;5:30

37. Altarescu GM, Goldfarb LG, Park KY, Kaneski C, Jeffries N, Litvak S, Nagle JW, Schiffmann R. Identification of fifteen novel mutations and genotype-phenotype relationship in fabry disease. *Clinical Genetics*. 2001;60:46-51

38. Blaydon D, Hill J, Winchester B. Fabry disease: 20 novel gla mutations in 35 families. *Human Mutation*. 2001;18

39. Ashley GA, Shabbeer J, Yasuda M, Eng CM, Desnick RJ. Fabry disease: Twenty novel alpha-galactosidase a mutations causing the classical phenotype. *J Human Genet*. 2001;46:192-196

40. Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH. Nature and frequency of mutations in the alpha-galactosidase a gene that cause fabry disease. *Am J Human Genet*. 1993;53:1186-1197

41. Shabbeer J, Yasuda M, Luca E, Desnick RJ. Fabry disease: 45 novel mutations in the alpha-galactosidase a gene causing the classical phenotype. *Mol Genet & Metab*. 2002;76:23-30

42. Pastores GM, Lien YH. Biochemical and molecular genetic basis of fabry disease. *J Am Soc Nephrol*. 2002;13 Suppl 2:S130-133

43. Redonnet-Vernhet I, Ploos van Amstel JK, Jansen RPM, Wevers RA, Salvayre R, Levade T. Uneven x inactivation in a female monozygotic twin pair with fabry disease and discordant expression of a novel mutation in the alpha-galactosidase a gene. *J Med Genet*. 1996;33:682-688

44. Mehta A, Ricci R, Widmer U, Dehout F, Garcia de Lorenzo A, Kampmann C, Linhart A, Sunder-Plassmann G, Ries M, Beck M. Fabry disease defined: Baseline clinical manifestations of 366 patients in the fabry outcome survey. *Eur J Clin Invest*. 2004;34:236-242

45. Sheth KJ, Werlin SL, Freeman ME, Hodach AE. Gastrointestinal structure and function in fabry's disease. *Am J Gastroenterol*. 1981;76:246-251

46. Shelley ED, Shelley WB, Kurczynski TW. Painful fingers, heat intolerance, and telangiectases of the ear: Easily ignored childhood signs of fabry disease. *Pediatr Dermatol*. 1995;12:215-219

47. Hilz MJ, Stemper B, Kolodny EH. Lower limb cold exposure induces pain and prolonged small fiber dysfunction in fabry patients. *Pain*. 2000;84:361-365

48. Mitsias P, Levine SR. Cerebrovascular complications of fabry's disease. *Ann Neurol*. 1996;40:8-17

49. Mendez MF, Stanley TM, Medel NM, Li Z, Tedesco DT. The vascular dementia of fabry's disease. *Dement Geriatr Cogn Disord*. 1997;8:252-257

50. DeGraba T, Azhar S, Dignat-George F, Brown E, Boutiere B, Altarescu G, McCarron R, Schiffmann R. Profile of endothelial and leukocyte activation in fabry patients. *Annals of Neurology*. 2000;47:229-233

51. Moore DF, Scott LT, Gladwin MT, Altarescu G, Kaneski C, Suzuki K, Pease-Fye M, Ferri R, Brady RO, Herscovitch P, Schiffmann R. Regional cerebral hyperperfusion and nitric oxide pathway dysregulation in fabry disease: Reversal by enzyme replacement therapy. *Circulation*. 2001;104:1506-1512

52. Moore DF, Altarescu G, Ling GS, Jeffries N, Frei KP, Weibel T, Charria-Ortiz G, Ferri R, Arai AE, Brady RO, Schiffmann R. Elevated cerebral blood flow velocities in fabry disease with reversal after enzyme replacement. *Stroke*. 2002;33:525-531

53. Sims K, Politei J, Banikazemi M, Lee P. Stroke in fabry disease frequently occurs before diagnosis and in the absence of other clinical events: Natural history data from the fabry registry. *Stroke*. 2009;40:788-794

54. O'Brien BD, Shnitka TK, McDougall R, Walker K, Costopoulos L, Lentle B, Anholt L, Freeman H, Thomson AB. Pathophysiologic and ultrastructural basis for intestinal symptoms in fabry's disease. *Gastroenterology*. 1982;82:957-962

55. Larralde M, Boggio P, Amartino H, Chamoles N. Fabry disease: A study of 6 hemizygous men and 5 heterozygous women with emphasis on dermatologic manifestations. *Arch Dermatol*. 2004;140:1440-1446

56. Mohrenschlager M, Henkel V, Ring J. Fabry disease: More than angiokeratomas. *Arch Dermatol*. 2004;140:1526-1528

57. Kanda A, Nakao S, Tsuyama S, Murata F, Kanzaki T. Fabry disease: Ultrastructural lectin histochemical analyses of lysosomal deposits. *Virchows Arch*. 2000;436:36-42

58. Orssaud C, Dufier J, Germain D. Ocular manifestations in fabry disease: A survey of 32 hemizygous male patients. *Ophthalmic Genet*. 2003;24:129-139

59. Alroy J, Sabnis S, Kopp JB. Renal pathology in fabry disease. *J Am Soc Nephrol*. 2002;13 Suppl 2:S134-138

60. Branton M, Schiffmann R, Kopp JB. Natural history and treatment of renal involvement in fabry disease. *J Am Soc Nephrol*. 2002;13 Suppl 2:S139-143

61. Desnick RJ, Blieden LC, Sharp HL, Hofschire PJ, Moller JH. Cardiac valvular anomalies in fabry disease. Clinical, morphologic, and biochemical studies. *Circulation*. 1976;54:818-825

62. Elleder M, Bradova V, Smid F, Budesinsky M, Harzer K, Kustermann-Kuhn B, Ledvinova J, Belohlavek, Kral V, Dorazilova V. Cardiocyte storage and hypertrophy as a sole manifestation of fabry's disease. Report on a case simulating hypertrophic non-obstructive cardiomyopathy. *Virchows Archiv A, Pathol Anat & Histopath*. 1990;417:449-455

63. Moon JC, Sachdev B, Elkington AG, McKenna WJ, Mehta A, Pennell DJ, Lee PJ, Elliott PM. Gadolinium enhanced cardiovascular magnetic resonance in

anderson-fabry disease. Evidence for a disease specific abnormality of the myocardial interstitium. *Eur Heart J*. 2003;24:2151-2155

64. Linhart A, Palecek T, Bultas J, Ferguson JJ, Hrudova J, Karetova D, Zeman J, Ledvinova J, Poupetova H, Elleder M, Aschermann M. New insights in cardiac structural changes in patients with fabry's disease. *Am Heart J*. 2000;139:1101-1108

65. von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hubner G, Olsen EG, Christomanou H, Kandolf R, Bishop DF, Desnick RJ. An atypical variant of fabry's disease with manifestations confined to the myocardium. *New Engl J Med*. 1991;324:395-399

66. Nagao Y, Nakashima H, Fukuhara Y, Shimmoto M, Oshima A, Ikari Y, Mori Y, Sakuraba H, Suzuki Y. Hypertrophic cardiomyopathy in late-onset variant of fabry disease with high residual activity of alpha-galactosidase a. *Clin Genet*. 1991;39:233-237

67. Ishii S, Kase R, Sakuraba H, Suzuki Y. Characterization of a mutant alpha-galactosidase gene product for the late-onset cardiac form of fabry disease. *Bioch Biophysic R Comm*. 1993;197:1585-1589

68. Kampmann C, Baehner F, Whybra C, Martin C, Wiethoff CM, Ries M, Gal A, Beck M. Cardiac manifestations of anderson-fabry disease in heterozygous females. *J Am Coll Cardiol*. 2002;40:1668-1674

69. Dobrovolny R, Dvorakova L, Ledvinova J, Magage S, Bultas J, Lubanda JC, Elleder M, Karetova D, Pavlikova M, Hrebicek M. Relationship between x-inactivation and clinical involvement in fabry heterozygotes. Eleven novel mutations in the

alpha-galactosidase a gene in the czech and slovak population. *J Mol Med (Berl)*. 2005;83:647-654

70. Desnick RJ, Dean KJ, Grabowski G, Bishop DF, Sweeley CC. Enzyme therapy in fabry disease: Differential in vivo plasma clearance and metabolic effectiveness of plasma and splenic alpha-galactosidase a isozymes. *Proc Natl Acad Sci U S A*. 1979;76:5326-5330

71. Schiffmann R, Murray GJ, Treco D, Daniel P, Sellos-Moura M, Myers M, Quirk JM, Zirzow GC, Borowski M, Loveday K, Anderson T, Gillespie F, Oliver KL, Jeffries NO, Doo E, Liang TJ, Kreps C, Gunter K, Frei K, Crutchfield K, Selden RF, Brady RO. Infusion of alpha-galactosidase a reduces tissue globotriaosylceramide storage in patients with fabry disease. *Proc Natl Acad Sci U S A*. 2000;97:365-370

72. Schiffmann R, Kopp JB, Austin HA, 3rd, Sabnis S, Moore DF, Weibel T, Balow JE, Brady RO. Enzyme replacement therapy in fabry disease: A randomized controlled trial.[comment]. *Jama.* 2001;285:2743-2749

73. Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ, International Collaborative Fabry Disease Study G. Safety and efficacy of recombinant human alpha-galactosidase a--replacement therapy in fabry's disease. *New Engl J Med.* 2001;345:9-16

74. Weidemann F, Breunig F, Beer M, Sandstede J, Turschner O, Voelker W, Ertl G, Knoll A, Wanner C, Strotmann JM. Improvement of cardiac function during enzyme replacement therapy in patients with fabry disease: A prospective strain rate imaging study. *Circulation*. 2003;108:1299-1301

75. Beck M, Ricci R, Widmer U, Dehout F, de Lorenzo AG, Kampmann C, Linhart A, Sunder-Plassmann G, Houge G, Ramaswami U, Gal A, Mehta A. Fabry disease: Overall effects of agalsidase alfa treatment. *Eur J Clin Invest*. 2004;34:838-844

76. Sheppard MN, Cane P, Florio R, Kavantzas N, Close L, Shah J, Lee P, Elliott P. A detailed pathologic examination of heart tissue from three older patients with anderson-fabry disease on enzyme replacement therapy. *Cardiovasc Pathol*. 2010;19:293-301

77. Kampmann C, Wiethoff CM, Martin C, Wenzel A, Kampmann R, Whybra C, Miebach E, Beck M. Electrocardiographic signs of hypertrophy in fabry disease-associated hypertrophic cardiomyopathy. *Acta Paediatrica Supplement.* 2002;91:21-27

78. Linhart A, Magage S, Palecek T, Bultas J. Cardiac involvement in fabry disease. *Acta Paediatrica Supplement.* 2002;91:15-20

79. Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, Elliott PM. Prevalence of anderson-fabry disease in male patients with late onset hypertrophic cardiomyopathy. *Circulation*. 2002;105:1407-1411

80. Romhilt DW, Estes EH, Jr. A point-score system for the ecg diagnosis of left ventricular hypertrophy. *Am Heart J*. 1968;75:752-758

81. Bellet S. *Clinical disorders of the heart beat*. Philadelphia: Lea & Febiger; 1971.

82. Fuster V, Ryden LE, Asinger RW, Cannom DS, Crijns HJ, Frye RL, Halperin JL, Kay GN, Klein WW, Levy S, McNamara RL, Prystowsky EN, Wann LS, Wyse DG, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Gregoratos G, Hiratzka LF, Jacobs AK, Russell RO, Smith SC, Alonso-Garcia A, Blomstrom-Lundqvi C, de Backer G, Flather M, Hradec J, Oto A, Parkhomenko A, Silber S, Torbicki A. Acc/aha/esc

guidelines for the management of patients with atrial fibrillation: Executive summary a report of the american college of cardiology/american heart association task force on practice guidelines and the european society of cardiology committee for practice guidelines and policy conferences (committee to develop guidelines for the management of patients with atrial fibrillation) developed in collaboration with the north american society of pacing and electrophysiology. *Circulation*. 2001;104:2118-2150

83. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: Comparison to necropsy findings. *Am J Cardiol*. 1986;57:450-458

84. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation*. 1977;55:613-618

85. Devereux RB, Liebson PR, Horan MJ. Recommendations concerning use of echocardiography in hypertension and general population research. *Hypertension*. 1987;9:II97-104

86. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, Singer DE. Prevalence of diagnosed atrial fibrillation in adults: National implications for rhythm management and stroke prevention: The anticoagulation and risk factors in atrial fibrillation (atria) study. *JAMA*. 2001;285:2370-2375

87. Utsumi K, Yamamoto N, Kase R, Takata T, Okumiya T, Saito H, Suzuki T, Uyama E, Sakuraba H. High incidence of thrombosis in fabry's disease. *Intern Med*. 1997;36:327-329

88. Grewal RP. Stroke in fabry's disease. *J Neurol*. 1994;241:153-156

89. Ritter M, Dittrich R, Droste DW. Microembolus detection in four patients with fabry's disease: Further support for a primarily microangiopathic origin of early cerebrovascular symptoms. *Eur Neurol*. 2003;50:141-145

90. Lenders M, Karabul N, Duning T, Schmitz B, Schelleckes M, Mesters R, Hense HW, Beck M, Brand SM, Brand E. Thromboembolic events in fabry disease and the impact of factor v leiden. *Neurology*. 2015;84:1009-1016

91. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, Hindricks G, Kirchhof P. 2012 focused update of the esc guidelines for the management of atrial fibrillation: An update of the 2010 esc guidelines for the management of atrial fibrillation. Developed with the special contribution of the european heart rhythm association. *Eur Heart J*. 2012;33:2719-2747

92. Sivaloganathan S. Fabry's disease--a rare cause of sudden death. *Med Sci Law*. 1992;32:263-266

93. Carter N, Milroy CM, Shepherd RT. Sudden death in elderly women with fabry's disease. *Am J Forensic Med Pathol*. 1995;16:21-26

94. Efthimiou J, McLelland J, Betteridge D. Short pr intervals and tachyarrhythmias in fabry's disease. *Postgrad Med J*. 1986;62:285-287

95. Eckart RE, Kinney KG, Belnap CM, Le TD. Ventricular fibrillation refractory to automatic internal cardiac defibrillator in fabry's disease. Review of cardiovascular manifestations. *Cardiology*. 2000;94:208-212

96. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, Yoshida A, Kuriyama M, Hayashibe H, Sakuraba H, et al. An atypical variant of fabry's disease in men with left ventricular hypertrophy. *New Engl J Med*. 1995;333:288-293

97. Ferrans VJ, Hibbs RG, Burda CD. The heart in fabry's disease. A histochemical and electron microscopic study. *Am J Cardiol*. 1969;24:95-110

98. Goldman ME, Cantor R, Schwartz MF, Baker M, Desnick RJ. Echocardiographic abnormalities and disease severity in fabry's disease. *J Am Coll Cardiol*. 1986;7:1157-1161

99. Bannwart F. [fabry's disease. Light and electron microscopic cardiac findings 12 years after successful kidney transplantation]. *Schweiz Med Wochenschr*. 1982;112:1742-1747

100. Broadbent JC, Edwards WD, Gordon H, Hartzler GO, Krawisz JE. Fabry cardiomyopathy in the female confirmed by endomyocardial biopsy. *Mayo Clin Proc*. 1981;56:623-628

101. Cantor WJ, Daly P, Iwanochko M, Clarke JT, Cusimano RJ, Butany J. Cardiac transplantation for fabry's disease. *Can J Cardiol*. 1998;14:81-84

102. Kramer W, Thormann J, Mueller K, Frenzel H. Progressive cardiac involvement by fabry's disease despite successful renal allotransplantation. *Int J Cardiol*. 1985;7:72-75

103. Maben P, Evans R, Lin J, Vaughan D. Endomyocardial biopsy. Diagnosis of fabry's disease in congestive cardiomyopathy. *J Kans Med Soc*. 1983;84:556-557

104. Heimdal A, Stoylen A, Torp H, Skjaerpe T. Real-time strain rate imaging of the left ventricle by ultrasound. *J Am Soc Echocardiogr*. 1998;11:1013-1019

105. Mehta A, Widmer U. Natural history of fabry disease. In: Mehta A, Beck M, Sunder-Plassman G, eds. *Fabry disease perspectives from 5 years of fos*. Oxford: PharmaGenesis; 2006:183-201.

106. Whybra C, Kampmann C, Krummenauer F, Ries M, Mengel E, Miebach E, Baehner F, Kim K, Bajbouj M, Schwarting A, Gal A, Beck M. The mainz severity score index: A new instrument for quantifying the anderson-fabry disease phenotype, and the response of patients to enzyme replacement therapy. *Clin Genet*. 2004;65:299-307

107. Beaver WL, Wasserman K, Whipp BJ. On-line computer analysis and breath-by-breath graphical display of exercise function tests. *J Appl Physiol*. 1973;34:128-132

108. Jones NL. Evaluation of a microprocessor-controlled exercise testing system. *J Appl Physiol*. 1984;57:1312-1318

109. Bruce RA, Kusumi F, Hosmer D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J*. 1973;85:546-562

110. Cooper DM, Weiler-Ravell D, Whipp BJ, Wasserman K. Aerobic parameters of exercise as a function of body size during growth in children. *J Appl Physiol*. 1984;56:628-634

111. Wasserman KH, Hansen JE, Sue DY, Whipp BJ, Casaburi R. *Principles of exercise testing and interpretation: Including pathophysiology and clinical applications*. London: Lippincott Williams & Wilkins; 1999.

112. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol*. 1986;60:2020-2027

113. Hansen JE, Sue DY, Wasserman K. Predicted values for clinical exercise testing. *Am Rev Respir Dis*. 1984;129:S49-55

114. Jones NL, Campbell EJM. *Clinical exercise testing*. Philadelphia: Saunders; 1988.

115. Cantor WJ, Butany J, Iwanochko M, Liu P. Restrictive cardiomyopathy secondary to fabry's disease. *Circulation*. 1998;98:1457-1459

116. Pieroni M, Chimenti C, Ricci R, Sale P, Russo MA, Frustaci A. Early detection of fabry cardiomyopathy by tissue doppler imaging. *Circulation*. 2003;107:1978-1984

117. Ikari Y, Kuwako K, Yamaguchi T. Fabry's disease with complete atrioventricular block: Histological evidence of involvement of the conduction system. *Br Heart J*. 1992;68:323-325

118. Galanos J, Nicholls K, Grigg L, Kiers L, Crawford A, Becker G. Clinical features of fabry's disease in australian patients. *Intern Med J*. 2002;32:575-584

119. Linhart A, Kampmann C, Zamorano JL, Sunder-Plassmann G, Beck M, Mehta A, Elliott PM. Cardiac manifestations of anderson-fabry disease: Results from the international fabry outcome survey. *Eur Heart J*. 2007;28:1228-1235

120. Aubert JD, Barbey F. Pulmonary involvement in fabry disease. In: Mehta A, Beck M, Sunder-Plassman G, eds. *Fabry disease perspectives from 5 years of fos*. Oxford: PharmaGenesis; 2006:263-270.

121. Shah JS, Lee P, Hughes D, Thaman R, Sachdev B, Pellerin D, Mehta A, Elliott PM. The natural history of left ventricular systolic function in anderson-fabry disease. *Heart*. 2005;91:533-534

122. Mills K, Vellodi A, Morris P, Cooper D, Morris M, Young E, Winchester B. Monitoring the clinical and biochemical response to enzyme replacement therapy in three children with fabry disease. *Eur J Pediatr*. 2004;163:595-603

123. Hermansen F, Rosen SD, Fath-Ordoubadi F, Kooner JS, Clark JC, Camici PG, Lammertsma AA. Measurement of myocardial blood flow with oxygen-15

labelled water: Comparison of different administration protocols. *Eur J Nucl Med*. 1998;25:751-759

124. Hermansen F, Ashburner J, Spinks TJ, Kooner JS, Camici PG, Lammertsma AA. Generation of myocardial factor images directly from the dynamic oxygen-15-water scan without use of an oxygen-15-carbon monoxide blood-pool scan. *J Nucl Med*. 1998;39:1696-1702

125. Elliott PM, Rosano GM, Gill JS, Poole-Wilson PA, Kaski JC, McKenna WJ. Changes in coronary sinus ph during dipyridamole stress in patients with hypertrophic cardiomyopathy. *Heart*. 1996;75:179-183

126. Camici P, Chiriatti G, Lorenzoni R, Bellina RC, Gistri R, Italiani G, Parodi O, Salvadori PA, Nista N, Papi L, et al. Coronary vasodilation is impaired in both hypertrophied and nonhypertrophied myocardium of patients with hypertrophic cardiomyopathy: A study with nitrogen-13 ammonia and positron emission tomography. *J Am Coll Cardiol*. 1991;17:879-886

127. Maron BJ, Wolfson JK, Epstein SE, Roberts WC. Intramural ("small vessel") coronary artery disease in hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 1986;8:545-557

128. Rajappan K, Rimoldi OE, Dutka DP, Ariff B, Pennell DJ, Sheridan DJ, Camici PG. Mechanisms of coronary microcirculatory dysfunction in patients with aortic stenosis and angiographically normal coronary arteries. *Circulation*. 2002;105:470-476

129. Rajappan K, Rimoldi OE, Camici PG, Bellenger NG, Pennell DJ, Sheridan DJ. Functional changes in coronary microcirculation after valve replacement in patients with aortic stenosis. *Circulation*. 2003;107:3170-3175

130. von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hubner G, Olsen EG, Christomanou H, Kandolf R, Bishop DF, Desnick RJ. An atypical variant of fabry's disease with manifestations confined to the myocardium. *N Engl J Med*. 1991;324:395-399

131. Altarescu G, Moore DF, Pursley R, Campia U, Goldstein S, Bryant M, Panza JA, Schiffmann R. Enhanced endothelium-dependent vasodilation in fabry disease. *Stroke*. 2001;32:1559-1562

132. Weidemann F, Breunig F, Beer M, Sandstede J, Turschner O, Voelker W, Ertl G, Knoll A, Wanner C, Strotmann JM. Improvement of cardiac function during enzyme replacement therapy in patients with fabry disease: A prospective strain rate imaging study. *Circulation*. 2003;108:1299-1301

133. Tayebjee MH, Lip GY, MacFadyen RJ. Matrix metalloproteinases in coronary artery disease:Clinical and therapeutic implications and pathological significance. . *Cur Medic Chem*. 2005;12:763-771

134. Tayebjee MH, Nadar S, Blann AD, Gareth Beevers D, MacFadyen RJ, Lip GY. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: A substudy of the anglo-scandinavian cardiac outcomes trial (ascot). *Am J Hypertens*. 2004;17:764-769

135. Tayebjee MH, Tan KT, MacFadyen RJ, Lip GYH. Abnormal circulating levels of metalloprotease 9 and its tissue inhibitor 1 in angiographically proven peripheral arterial disease: Relationship to disease severity. *J Intern Med*. 2005;257:110-116

136. Tayebjee MH, Nadar SK, MacFadyen RJ, Lip GY. Tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-9 levels in patients with hypertension relationship to tissue doppler indices of diastolic relaxation. *Am J Hypertens*. 2004;17:770-774

137. Lindsay MM, Maxwell P, Dunn FG. Timp-1: A marker of left ventricular diastolic dysfunction and fibrosis in hypertension. *Hypertension*. 2002;40:136-141

138. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. *J Biol Chem*. 1999;274:21491-21494

139. McCawley LJ, Matrisian LM. Matrix metalloproteinases: They're not just for matrix anymore! *Curr Opin Cell Biol*. 2001;13:534-540

140. McDonnell S, Morgan M, Lynch C. Role of matrix metalloproteinases in normal and disease processes. *Biochem Soc Trans*. 1999;27:734-740

141. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: Biologic activity and clinical implications. *J Clin Oncol*. 2000;18:1135-1149

142. Inokubo Y, Hanada H, Ishizaka H, Fukushi T, Kamada T, Okumura K. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. *Am Heart J*. 2001;141:211-217

143. Kaden JJ, Dempfle CE, Sueselbeck T, Brueckmann M, Poerner TC, Haghi D, Haase KK, Borggrefe M. Time-dependent changes in the plasma concentration of matrix metalloproteinase 9 after acute myocardial infarction. *Cardiology*. 2003;99:140-144

144. Altieri P, Brunelli C, Garibaldi S, Nicolino A, Ubaldi S, Spallarossa P, Olivotti L, Rossettin P, Barsotti A, Ghigliotti G. Metalloproteinases 2 and 9 are increased in plasma of patients with heart failure. *Eur J Clin Invest*. 2003;33:648-656

145. Reinhardt D, Sigusch HH, Hensse J, Tyagi SC, Korfer R, Figulla HR. Cardiac remodelling in end stage heart failure: Upregulation of matrix metalloproteinase (mmp) irrespective of the underlying disease, and evidence for a direct inhibitory effect of ace inhibitors on mmp. *Heart*. 2002;88:525-530

146. Sundstrom J, Evans JC, Benjamin EJ, Levy D, Larson MG, Sawyer DB, Siwik DA, Colucci WS, Sutherland P, Wilson PWF, Vasan RS. Relations of plasma matrix metalloproteinase-9 to clinical cardiovascular risk factors and echocardiographic left ventricular measures: The framingham heart study. *Circulation*. 2004;109:2850-2856

147. Stetler-Stevenson WG, Seo DW. Timp-2: An endogenous inhibitor of angiogenesis. *Trends Mol Med*. 2005;11:97-103

148. Dolgilevich SM, Siri FM, Atlas SA, Eng C. Changes in collagenase and collagen gene expression after induction of aortocaval fistula in rats. *Am J Physiol Heart Circ Physiol*. 2001;281:H207-214

149. Linhart A, Lubanda JC, Palecek T, Bultas J, Karetova D, Ledvinova J, Elleder M, Aschermann M. Cardiac manifestations in fabry disease. *J Inherit Metab Dis*. 2001;24 Suppl 2:75-83; discussion 65

150. Lobo T, Morgan J, Bjorksten A, Nicholls K, Grigg L, Centra E, Becker G. Cardiovascular testing in fabry disease: Exercise capacity reduction, chronotropic incompetence and improved anaerobic threshold after enzyme replacement. *Intern Med J*. 2008;38:407-414

151. Kampmann C, Wiethoff CM, Whybra C, Baehner FA, Mengel E, Beck M. Cardiac manifestations of anderson-fabry disease in children and adolescents. *Acta Paediatr*. 2008;97:463-469

152. O'Mahony C, Coats C, Cardona M, Garcia A, Calcagnino M, Murphy E, Lachmann R, Mehta A, Hughes D, Elliott PM. Incidence and predictors of anti-bradycardia pacing in patients with anderson-fabry disease. *Europace*. 2011;13:1781-1788

153. Frustaci A, Chimenti C. Images in cardiovascular medicine. Cryptogenic ventricular arrhythmias and sudden death by fabry disease: Prominent infiltration of cardiac conduction tissue. *Circulation*. 2007;116:e350-351

154. Shah JS, Hughes DA, Sachdev B, Tome M, Ward D, Lee P, Mehta AB, Elliott PM. Prevalence and clinical significance of cardiac arrhythmia in anderson-fabry disease. *Am J Cardiol*. 2005;96:842-846

155. Weidemann F, Niemann M, Stork S, Breunig F, Beer M, Sommer C, Herrmann S, Ertl G, Wanner C. Long-term outcome of enzyme-replacement therapy in advanced fabry disease: Evidence for disease progression towards serious complications. *J Intern Med*. 2013;274:331-341

156. Mehta AB. Fabry disease: Is there a role for enzyme replacement therapy? *J Intern Med*. 2013;274:329-330

157. Frustaci A, Morgante E, Russo MA, Scopelliti F, Grande C, Verardo R, Franciosa P, Chimenti C. Pathology and function of conduction tissue in fabry disease cardiomyopathy. *Circ Arrhythm Electrophysiol*. 2015

158. Moon JC, Sheppard M, Reed E, Lee P, Elliott PM, Pennell DJ. The histological basis of late gadolinium enhancement cardiovascular magnetic resonance in a

patient with anderson-fabry disease. *J Cardiovasc Magn Reson*. 2006;8:479-482

159. Palecek T, Dostalova G, Kuchynka P, Karetova D, Bultas J, Elleder M, Linhart A. Right ventricular involvement in fabry disease. *J Am Soc Echocardiogr*. 2008;21:1265-1268

160. Kampmann C, Baehner FA, Whybra C, Bajbouj M, Baron K, Knuf M, Wiethoff CM, Trubel H, Beck M. The right ventricle in fabry disease. *Acta Paediatr Suppl*. 2005;94:15-18; discussion 19-10

161. Chimenti C, Hamdani N, Boontje NM, DeCobelli F, Esposito A, Bronzwaer JG, Stienen GJ, Russo MA, Paulus WJ, Frustaci A, van der Velden J. Myofilament degradation and dysfunction of human cardiomyocytes in fabry disease. *Am J Pathol*. 2008;172:1482-1490

162. Weidemann F, Niemann M, Breunig F, Herrmann S, Beer M, Stork S, Voelker W, Ertl G, Wanner C, Strotmann J. Long-term effects of enzyme replacement therapy on fabry cardiomyopathy: Evidence for a better outcome with early treatment. *Circulation*. 2009;119:524-529

163. Pieroni M, Chimenti C, De Cobelli F, Morgante E, Del Maschio A, Gaudio C, Russo MA, Frustaci A. Fabry's disease cardiomyopathy: Echocardiographic detection of endomyocardial glycosphingolipid compartmentalization. *J Am Coll Cardiol*. 2006;47:1663-1671

164. Kounas S, Demetrescu C, Pantazis AA, Keren A, Lee PJ, Hughes D, Mehta A, Elliott PM. The binary endocardial appearance is a poor discriminator of anderson-fabry disease from familial hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2008;51:2058-2061

165. Bierer G, Kamangar N, Balfe D, Wilcox WR, Mosenifar Z. Cardiopulmonary exercise testing in fabry disease. *Respiration*. 2005;72:504-511

166. Bierer G, Balfe D, Wilcox WR, Mosenifar Z. Improvement in serial cardiopulmonary exercise testing following enzyme replacement therapy in fabry disease. *J Inherit Metab Dis*. 2006;29:572-579

167. Spinelli L, Nicolai E, Acampa W, Imbriaco M, Pisani A, Rao MA, Scopacasa F, Cianciaruso B, De Luca N, Cuocolo A. Cardiac performance during exercise in patients with fabry's disease. *Eur J Clin Invest*. 2008;38:910-917

168. Kalliokoski RJ, Kantola I, Kalliokoski KK, Engblom E, Sundell J, Hannukainen JC, Janatuinen T, Raitakari OT, Knuuti J, Penttinen M, Viikari J, Nuutila P. The effect of 12-month enzyme replacement therapy on myocardial perfusion in patients with fabry disease. *J Inherit Metab Dis*. 2006;29:112-118

169. Kalliokoski RJ, Kalliokoski KK, Sundell J, Engblom E, Penttinen M, Kantola I, Raitakari OT, Knuuti J, Nuutila P. Impaired myocardial perfusion reserve but preserved peripheral endothelial function in patients with fabry disease. *J Inherit Metab Dis*. 2005;28:563-573

170. Kovarnik T, Mintz GS, Karetova D, Horak J, Bultas J, Skulec R, Skalicka H, Aschermann M, Elleder M, Linhart A. Intravascular ultrasound assessment of coronary artery involvement in fabry disease. *J Inherit Metab Dis*. 2008;31:753-760

171. Barbey F, Brakch N, Linhart A, Rosenblatt-Velin N, Jeanrenaud X, Qanadli S, Steinmann B, Burnier M, Palecek T, Bultas J, Hayoz D. Cardiac and vascular hypertrophy in fabry disease: Evidence for a new mechanism independent of

blood pressure and glycosphingolipid deposition. *Arterioscler Thromb Vasc Biol*. 2006;26:839-844

172. Chimenti C, Morgante E, Tanzilli G, Mangieri E, Critelli G, Gaudio C, Russo MA, Frustaci A. Angina in fabry disease reflects coronary small vessel disease. *Circ Heart Fail*. 2008;1:161-169

173. Kitaoka H, Kubo T, Okawa M, Hayato K, Yamasaki N, Matsumura Y, Doi YL. Impact of metalloproteinases on left ventricular remodeling and heart failure events in patients with hypertrophic cardiomyopathy. *Circ J*. 2010;74:1191-1196

174. Coats CJ, Parisi V, Ramos M, Janagarajan K, O'Mahony C, Dawnay A, Lachmann RH, Murphy E, Mehta A, Hughes D, Elliott PM. Role of serum n-terminal pro-brain natriuretic peptide measurement in diagnosis of cardiac involvement in patients with anderson-fabry disease. *Am J Cardiol*. 2013;111:111-117

175. De Cobelli F, Esposito A, Belloni E, Pieroni M, Perseghin G, Chimenti C, Frustaci A, Del Maschio A. Delayed-enhanced cardiac mri for differentiation of fabry's disease from symmetric hypertrophic cardiomyopathy. *AJR Am J Roentgenol*. 2009;192:W97-102

176. Beer M, Weidemann F, Breunig F, Knoll A, Koeppe S, Machann W, Hahn D, Wanner C, Strotmann J, Sandstede J. Impact of enzyme replacement therapy on cardiac morphology and function and late enhancement in fabry's cardiomyopathy. *Am J Cardiol*. 2006;97:1515-1518

177. Aerts JM, Groener JE, Kuiper S, Donker-Koopman WE, Strijland A, Ottenhoff R, van Roomen C, Mirzaian M, Wijburg FA, Linthorst GE, Vedder AC, Rombach SM, Cox-Brinkman J, Somerharju P, Boot RG, Hollak CE, Brady RO, Poorthuis BJ.

Elevated globotriaosylsphingosine is a hallmark of fabry disease. *Proc Natl Acad Sci U S A*. 2008;105:2812-2817

178. Shen JS, Meng XL, Moore DF, Quirk JM, Shayman JA, Schiffmann R, Kaneski CR. Globotriaosylceramide induces oxidative stress and up-regulates cell adhesion molecule expression in fabry disease endothelial cells. *Mol Genet Metab*. 2008;95:163-168

179. Ashrafian H, Redwood C, Blair E, Watkins H. Hypertrophic cardiomyopathy:A paradigm for myocardial energy depletion. *Trends Genet*. 2003;19:263-268

180. Jung WI, Sieverding L, Breuer J, Hoess T, Widmaier S, Schmidt O, Bunse M, van Erckelens F, Apitz J, Lutz O, Dietze GJ. 31p nmr spectroscopy detects metabolic abnormalities in asymptomatic patients with hypertrophic cardiomyopathy. *Circulation*. 1998;97:2536-2542

181. Lucke T, Hoppner W, Schmidt E, Illsinger S, Das AM. Fabry disease: Reduced activities of respiratory chain enzymes with decreased levels of energy-rich phosphates in fibroblasts. *Mol Genet Metab*. 2004;82:93-97

182. Linthorst GE, Bouwman MG, Wijburg FA, Aerts JM, Poorthuis BJ, Hollak CE. Screening for fabry disease in high-risk populations: A systematic review. *J Med Genet*. 2010;47:217-222

183. Rombach SM, Smid BE, Bouwman MG, Linthorst GE, Dijkgraaf MG, Hollak CE. Long term enzyme replacement therapy for fabry disease: Effectiveness on kidney, heart and brain. *Orphanet J Rare Dis*. 2013;8:47

184. El Dib RP, Nascimento P, Pastores GM. Enzyme replacement therapy for anderson-fabry disease. *Cochrane Database Syst Rev*. 2013;2:CD006663

185. Keslova-Veselikova J, Hulkova H, Dobrovolny R, Asfaw B, Poupetova H, Berna L, Sikora J, Golan L, Ledvinova J, Elleder M. Replacement of alpha-galactosidase a in fabry disease: Effect on fibroblast cultures compared with biopsied tissues of treated patients. *Virchows Arch*. 2008;452:651-665

186. Murray GJ, Anver MR, Kennedy MA, Quirk JM, Schiffmann R. Cellular and tissue distribution of intravenously administered agalsidase alfa. *Mol Genet Metab*. 2007;90:307-312

187. Frustaci A, Chimenti C, Ricci R, Natale L, Russo MA, Pieroni M, Eng CM, Desnick RJ. Improvement in cardiac function in the cardiac variant of fabry's disease with galactose-infusion therapy. *New Engl J Med*. 2001;345:25-32

188. Ries M, Ramaswami U, Parini R, Lindblad B, Whybra C, Willers I, Gal A, Beck M. The early clinical phenotype of fabry disease: A study on 35 european children and adolescents. *Eur J Pediatr*. 2003;162:767-772

189. Ramaswami U, Parini R, Pintos-Morell G. In: Mehta a, beck m, sunder-plassmann g, editors. Fabry disease: Perspectives from 5 years of fos. Oxford: Oxford pharmagenesis. Natural history and effects of enzyme replacement therapy in children and adolescents with fabry disease. Chapter 31. 2006.

190. Ramaswami U. Fabry disease during childhood: Clinical manifestations and treatment with agalsidase alfa. *Acta Paediatr Suppl*. 2008;97:38-40

191. Vedder AC, Linthorst GE, Houge G, Groener JE, Ormel EE, Bouma BJ, Aerts JM, Hirth A, Hollak CE. Treatment of fabry disease: Outcome of a comparative trial with agalsidase alfa or beta at a dose of 0.2 mg/kg. *PLoS One*. 2007;2:e598

192. Imbriaco M, Pisani A, Spinelli L, Cuocolo A, Messalli G, Capuano E, Marmo M, Liuzzi R, Visciano B, Cianciaruso B, Salvatore M. Effects of enzyme-replacement

therapy in patients with anderson-fabry disease: A prospective long-term cardiac magnetic resonance imaging study. *Heart*. 2009;95:1103-1107

193. Hughes DA, Elliott PM, Shah J, Zuckerman J, Coghlan G, Brookes J, Mehta AB. Effects of enzyme replacement therapy on the cardiomyopathy of anderson-fabry disease: A randomised, double-blind, placebo-controlled clinical trial of agalsidase alfa. *Heart*. 2008;94:153-158

194. Baehner F, Kampmann C, Whybra C, Miebach E, Wiethoff CM, Beck M. Enzyme replacement therapy in heterozygous females with fabry disease: Results of a phase iiib study. *J Inherit Metab Dis*. 2003;26:617-627

195. Rombach SM, Smid BE, Linthorst GE, Dijkgraaf MG, Hollak CE. Natural course of fabry disease and the effectiveness of enzyme replacement therapy: A systematic review and meta-analysis: Effectiveness of ert in different disease stages. *J Inherit Metab Dis*. 2014;37:341-352

196. Germain DP, Giugliani R, Hughes DA, Mehta A, Nicholls K, Barisoni L, Jennette CJ, Bragat A, Castelli J, Sitaraman S, Lockhart DJ, Boudes PF. Safety and pharmacodynamic effects of a pharmacological chaperone on alpha-galactosidase a activity and globotriaosylceramide clearance in fabry disease: Report from two phase 2 clinical studies. *Orphanet J Rare Dis*. 2012;7:91

197. Shin SH, Kluepfel-Stahl S, Cooney AM, Kaneski CR, Quirk JM, Schiffmann R, Brady RO, Murray GJ. Prediction of response of mutated alpha-galactosidase a to a pharmacological chaperone. *Pharmacogenet Genomics*. 2008;18:773-780

198. Cox T, Lachmann R, Hollak C, Aerts J, van Weely S, Hrebicek M, Platt F, Butters T, Dwek R, Moyses C, Gow I, Elstein D, Zimran A. Novel oral treatment of

gaucher's disease with n-butyldeoxynojirimycin (ogt 918) to decrease substrate biosynthesis. *Lancet*. 2000;355:1481-1485

199. Abe A, Gregory S, Lee L, Killen PD, Brady RO, Kulkarni A, Shayman JA. Reduction of globotriaosylceramide in fabry disease mice by substrate deprivation. *J Clin Invest*. 2000;105:1563-1571

200. Ohshima T, Murray GJ, Swaim WD, Longenecker G, Quirk JM, Cardarelli CO, Sugimoto Y, Pastan I, Gottesman MM, Brady RO, Kulkarni AB. Alpha-galactosidase a deficient mice: A model of fabry disease. *Proc Natl Acad Sci U S A*. 1997;94:2540-2544

201. Jung SC, Han IP, Limaye A, Xu R, Gelderman MP, Zerfas P, Tirumalai K, Murray GJ, During MJ, Brady RO, Qasba P. Adeno-associated viral vector-mediated gene transfer results in long-term enzymatic and functional correction in multiple organs of fabry mice. *Proc Natl Acad Sci U S A*. 2001;98:2676-2681

202. Park J, Murray GJ, Limaye A, Quirk JM, Gelderman MP, Brady RO, Qasba P. Long-term correction of globotriaosylceramide storage in fabry mice by recombinant adeno-associated virus-mediated gene transfer. *Proc Natl Acad Sci U S A*. 2003;100:3450-3454

203. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, Brugman MH, Pike-Overzet K, Chatters SJ, de Ridder D, Gilmour KC, Adams S, Thornhill SI, Parsley KL, Staal FJ, Gale RE, Linch DC, Bayford J, Brown L, Quaye M, Kinnon C, Ancliff P, Webb DK, Schmidt M, von Kalle C, Gaspar HB, Thrasher AJ. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of scid-x1 patients. *J Clin Invest*. 2008;118:3143-3150

204. Enquist IB, Nilsson E, Ooka A, Mansson JE, Olsson K, Ehinger M, Brady RO, Richter J, Karlsson S. Effective cell and gene therapy in a murine model of gaucher disease. *Proc Natl Acad Sci U S A*. 2006;103:13819-13824

Publications arising from dissertation

Shah JS, Hughes DA, Sachdev B, Tome M, Ward D, Lee P, Mehta AB, Elliott PM. Prevalence and clinical significance of cardiac arrhythmia in Anderson-Fabry disease. *Am J Cardiol.* 2005; 96(6):842-846.

Shah JS, Lee P, Hughes D, Thaman R, Sachdev B, Pellerin D, Mehta A, Elliott PM. The natural history of left ventricular systolic function in Anderson-Fabry disease. *Heart.* 2005; 91(4):533-534.

Shah JS, Elliott PM. Fabry disease and the heart: an overview of the natural history and the effect of enzyme replacement therapy. *Acta Paediatr Suppl.* Mar 2005; 94(447):11-14; discussion 19-10.

Elliott PM, Kindler H, Shah JS, Sachdev B, Rimoldi O, Thaman R, Tome MT, McKenna WJ, Lee P, Camici PG. Coronary microvascular dysfunction in male patients with Anderson-Fabry disease and the effect of treatment with alpha galactosidase A. *Heart.* 2006;92(3):357-60

Shah JS, Hughes DA, Tayebjee MH, MacFadyen RJ, Mehta AB, Elliott PM. Extracellular matrix turnover and disease severity in Anderson-Fabry disease. *J Inherit Metab Dis.* 2007; 30(1):88-95.

Sheppard MN, Cane P, Florio R, Kavantzas N, Close L, Shah J, Lee P, Elliott P. A detailed pathologic examination of heart tissue from three older patients with anderson-fabry disease on enzyme replacement therapy. *Cardiovasc Pathol*. 2010;19:293-301