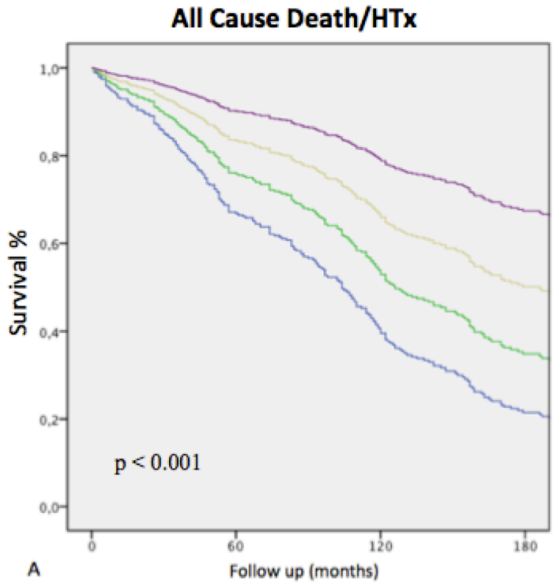
The presence of such favourable clinical profile resulted in a better outcome, so that each enrollment period was associated with a 42% reduction in risk of all-cause mortality and transplantation, compared with the previous one [Figure 4.3-2 A]. Likewise, an earlier period of enrollment proved a potent independent predictor of refractory HF death and SD [Figure 4.3-2 B,C].

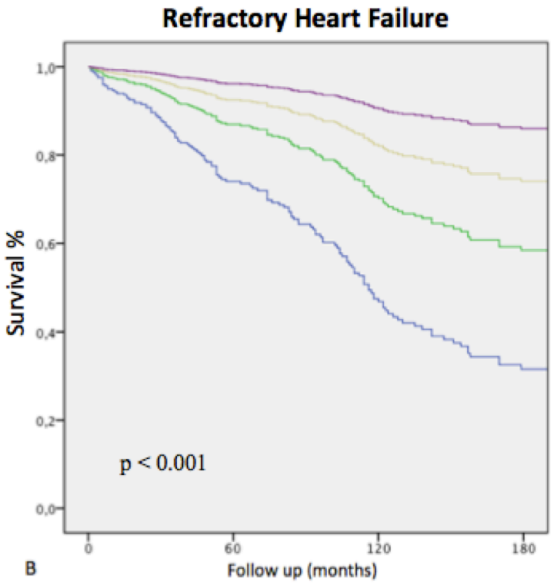
HF death rates declined at a relatively constant rate after the introduction of ACE inhibition, in the transition from periods 1 to 2. Conversely, marked and progressive impact on SD was evident in the transition to periods 3 and 4, after the introduction of the ICD/CRT and the complete penetration of β-blocker treatment for HF in real-world practice.

Figure 4.3-2 (following page)

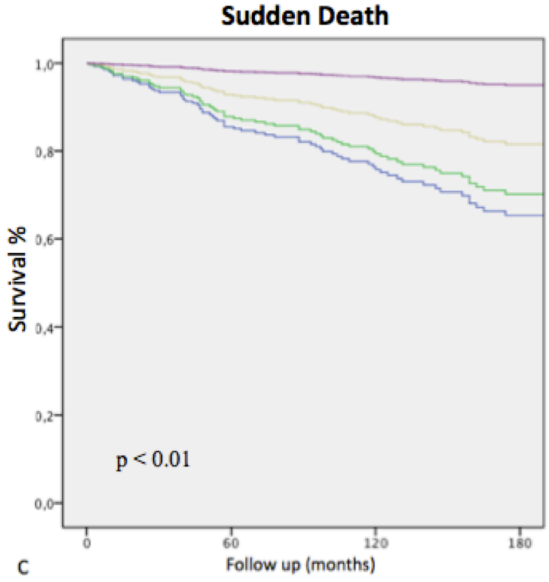
Cox multivariate regression survival plot (adjusted for age, New York Heart Association [NYHA] class, sex, left ventricular ejection fraction and indexed left atrium diameter) indicating differences in outcome based on enrollment period for the 3 study end points: **A**, All-cause mortality/heart transplantation; (**B**) death for refractory heart failure; and (**C**) sudden death.


All Cause Death/HTx
Survival %
1,0
0,8
0,6
0,4
0,2
0,0
p < 0.001
0
60
120
180
Follow up (months)

A


Refractory Heart Failure
Survival %
1,0
0,8
0,6
0,4
0,2
0,0
p < 0.001
0
60
120
180
Follow up (months)

B


Sudden Death
Survival %
1,0
0,8
0,6
0,4
0,2
0,0
p < 0.01
0
60
120
180
Follow up (months)

C

It is important to notice that in our cohort, patients enrolled later had a less severe profile in terms of symptoms, LV size and function. This trend presumably reflects increasing awareness of the disease in the cardiology community and availability of more sensitive diagnostic tools that allowed an early diagnosis of the disease. This may have contributed to the reduction in mortality observed during the years, resulting in an overestimation of benefits related to treatment. However, this trend is counterbalanced by the more advanced age at enrollment of the most recent groups. In our multivariate models, the enrolment period proved to be a very potent predictor of outcome independent of the most relevant baseline clinical and demographic features including well-recognized prognostic factors (ie, age, sex, NYHA functional class, LV and left atrial diameters, LVEF, and degree of functional mitral regurgitation) [50-54]. Although it is virtually impossible to quantify the net result of these trends on outcome, it is plausible to attribute most of the survival benefit observed to improvements in management rather than to the evolving patient demographics.

4.3.3 Conclusions

The present study demonstrates that the long-term prognosis of patients with idiopathic dilated cardiomyopathy has radically improved during the past 30 years, in terms of overall mortality, refractory heart failure death, and sudden cardiac death, reflecting continuing progress in pharmacological and device-based management.

The power of our data goes well beyond the huge number of highly selected patients, the extend period of follow-up and the stability of the

team-care over time. It indeed resides in the possibility to replicate the setting of a clinical trial in the real world, overcoming those limitations usually associated with purely retrospective and observational community studies with long follow-up where data are not usually collected in a systematic fashion.

4.4 Myocardial damage due to infectious disease: Chagas cardiomyopathy

Patients suffering from spatial neglect may eat from only one side of a plate, write on only one side of a page, or shave only one side of the face. The neglect may be so strong so as to deny ownership of the other side, stating that it belongs to someone else, or to avoid or withdrawn from the contralateral side of space.

There is a form of special neglect affecting the medical community: more than one billion of people worldwide suffer from a group of infections caused by a variety of pathogens, responsible for a significant numbers of death in tropical and sub-tropical areas. However, the majority of these tropical diseases afflict the poorest people, who live on less than $2 per day [55]. Therefore, despite their significance in terms of burden of ill carried by more than one-sixth of the world's population, little financial support has been provided to address these diseases, like they don't even exist. That is the reason why the World Health Organization decided to unify them under the name of “neglected tropical diseases” and prioritize 17 of them in an official list, in order to increase their awareness among physicians of the eastern countries [55-57]. Moreover, due to the increasing immigration, international travel and adoption, many of these diseases now represent a matter of international concern even for non-endemic countries, such as Europe or US [58-60]. They are a ‘neglect’ too important to ignore.

We started to explore this “new” branch of cardiology in 2011, as a result of our collaboration with the Infectious Disease Unit of our Hospital. Two of young women with the chronic form of Chagas disease were referred to our center for cardiologic evaluation. They showed heart

failure due to dilated cardiomyopathy and isolated ventricular arrhythmias as dominant features of cardiac involvement, which was substantially comparable to other more common form of heart disease.

Therefore, we decided to share our experience describing the story of two patients with CD, in order to emphasizes the role of Chagas disease as an important cause of cardiac disease in patients from endemic regions, even when long removed from their native countries, highlighting the need for increased awareness in non-endemic settings.

4.4.1 Clinical pictures

Patient # 1:

A 46-year old Brazilian woman, who has resided in Italy for 24 years, was admitted to our hospital following the onset of worsening dyspnea and cough over the previous 3 months. The patient had a longstanding history of smoking (1 pack/day), but no other known cardiovascular risk factors. Two months previously, she had been seen by a pneumologist, and had a chest CT and pulmonary function tests, upon which a tentative diagnosis of interstitial pulmonary disease was made. Treatment with oral steroids had been started over the ensuing 4 weeks, with little benefit. On admission, she complained of shortness of breath during daily activities and was unable to climb stairs (NYHA functional class III), but had no history of chest pain or palpitations. On examination, she had a 3rd heart sound but no murmurs; JVP was slightly elevated, and she had fine bilateral basal crackles on chest auscultation. A repeat CT of her chest showed evidence of pulmonary congestion, with mild interstitial thickening; pulmonary function tests were consistent with moderate restrictive respiratory impairment. Her electrocardiogram (ECG) showed

sinus rhythm with occasional ventricular ectopic beats, but was otherwise unremarkable. A transthoracic echocardiogram, however, showed a mildly dilated, diffusely hypokinetic left ventricle (LV), with an ejection fraction of 38% and severe functional mitral regurgitation. Routine laboratory tests and standard virology, including cardiotropic and neurologic agents, were inconclusive. A normal coronary angiogram excluded ischemic heart disease as a cause of LV dysfunction.

When other potential etiologies were explored, a detailed family history revealed that the patient's sister had died suddenly years before in Brazil with a prior diagnosis of Chagas, prompting specific investigations for T. Cruzi. Serology tests resulted positive, leading to the diagnosis of chronic CD complicated by cardiomyopathy.

Standard treatment for heart failure was begun with carvedilol, ramipril and loop diuretics. Following multidisciplinary consultation, antitrypanosmomal therapy was felt not indicated at this stage and the patient was discharged in stable conditions.

After one month, her dyspnea had progressively improved and she remained in NYHA functional class II. However, she complained of recurrent palpitations and a 24-hour ambulatory Holter ECG showed extremely frequent polymorphic premature ventricular beats, with several couples and triplets. Amiodarone was added to her therapeutic regimen at a dose of 200 mg/day.

At 3 months, she reported further improvement, with only mild exercise limitation and no further episodes of palpitations. A repeat Holter ECG showed dramatic reduction in the frequency of ventricular and supraventricular beats. Her echocardiogram had also improved, with significant increment in LV ejection fraction (to 52%) and only mild residual mitral regurgitation [Figure 4.4-1]. Cardiac MRI showed discrete

LV akinesia localized to the apical region, apical septum and infero-lateral wall; following gadolinium injection, there was evident delayed enhancement of the apex with prevalent sub-epicardial distribution, consistent with Chagas cardiomyopathy [Figure 4.4-2].

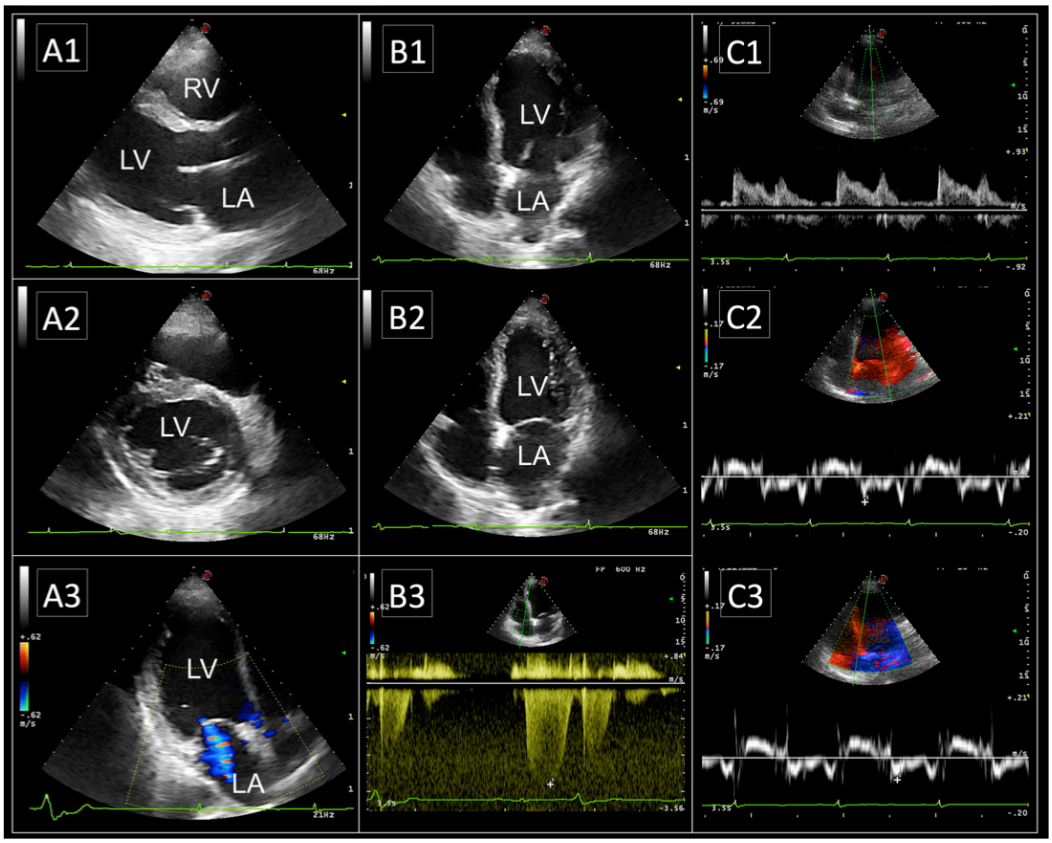


Figure 4.4-1: Transthoracic echocardiogram from patient #1.

Parasternal long axis and short axis views (panel A1 and panel A2) and apical four chamber view (panel B1 and panel B2), showed mild left ventricular dilatation and dysfunction, with functional mitral regurgitation (panel A3) and moderate tricuspid regurgitation (panel B3). A triphasic pattern of left ventricular diastolic filling recorded with pulsed Doppler (panel C1) associated with reduced tissue Doppler lateral (panel C2) and septal E' (panel C3), consistent with moderate diastolic dysfunction.

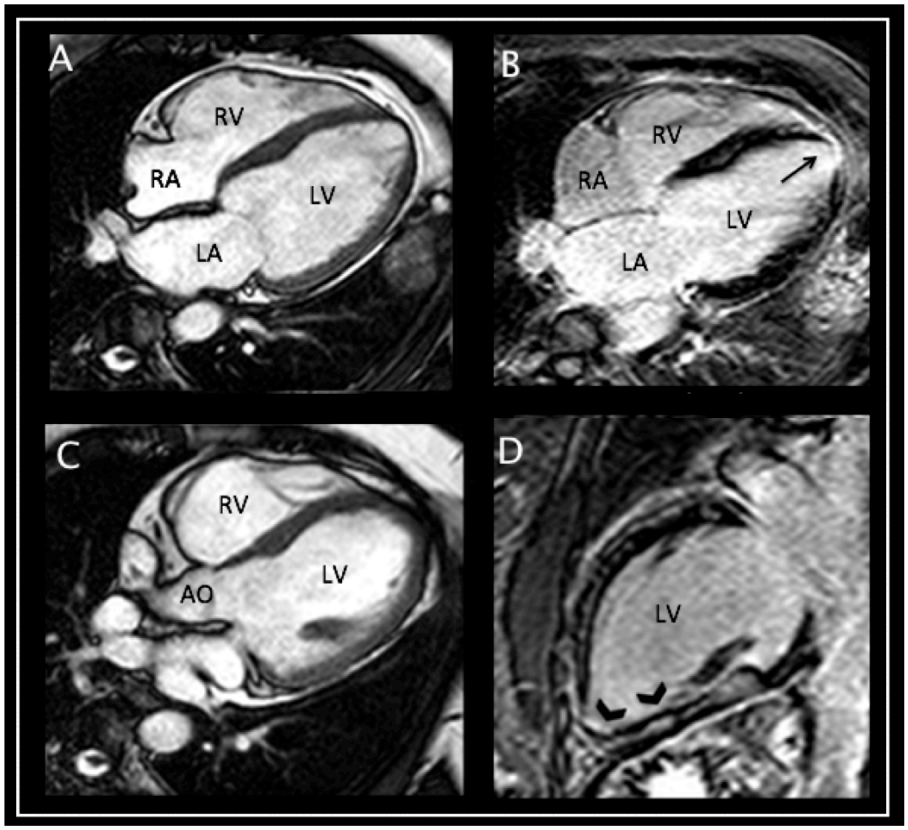


Figure 4.4-2: Cardiac MRI from patient #1.

Four-chamber enlargement is accompanied by a-dyskinesia of apical region, distal septum and infero-lateral wall (panel A-C), associated with transmurallate gadolinium enhancement of the apical region (arrow) (panel B), with sub-epicardial distribution evident in two-chamber view (arrowhead) (panel D).

After one year of optimal medical therapy, the patient was in stable clinical conditions, with no symptoms except for occasional fatigue. An implantable cardioverter defibrillator (ICD) was discussed with the patient, who declined based on her excellent response to medical treatment. At this stage, antitrypanosomal treatment with benznidazole was introduced but had to be withdrawn on day 12 due to a diffuse itching maculopapular skin rash, which worsened despite oral treatment with hydroxyzine dihydrochloride and prednisone. Benznidazole was

therefore discontinued and no further antytripanosomal treatment has been considered so far. She's currently been considered for nifurtimox.

One year later, during a scheduled follow-up visit, she complained of palpitations, insomnia and anxiety, started a couple of weeks earlier. The ECG showed frequent isolated ventricular ectopic beats, while LV function was stable. Blood tests revealed a severe hyperthyroidism probably related to amiodarone, with T3 level about 100 times higher than the normal range. Therefore, methimazole was started with progressive reduction of thyroids hormones level and clinical improvement. Amiodarone was immediately stopped, but no other antiarrhythmic drugs could be considered because of the underlying cardiac condition. Thus, after a careful reevaluation of risk factor for sudden cardiac death, the ICD was reconsidered and then implanted.

Patient #2

A 48-year old Bolivian woman, resident in Italy for 32 years, was referred for cardiac evaluation by the infectious disease unit after a recent diagnosis of CD, following screening of the local Bolivian Community in central Italy. Her father and sister had both died at a young age, possibly due to complications of CD. She complained of recurrent exertional chest discomfort, but no dyspnoea or palpitations. On examination she was found to have an irregular pulse, while the rest of her examination was otherwise unremarkable. A transthoracic echocardiogram showed normal LV function with trivial mitral regurgitation and normal pulmonary pressures. However, her 24-hours Holter ECG showed numerous monomorphic premature ventricular beats with frequent periods of bigeminy and trigeminy. A symptom-limited, maximal exercise test ruled out inducible ischemia. Treatment with

amiodarone was started at the dose of 200 mg/day followed after one month by antiparasitic treatment with benznidazole 150 mg twice daily for 66 days. The latter was well tolerated. At 2 months, a repeat Holter ECG showed marked reduction of the arrhythmic burden, an improvement paralleled by relief of her exertional chest discomfort. At one year, she was asymptomatic on amiodarone, with no evidence of structural heart disease on her echocardiogram.

4.4.2 The kissing bug

Chagas disease (CD), also known as American Trypanosomiasis, is a parasitic disease caused by the protozoan *Trypanosoma cruzi*, endemic to Central and South America, where 16-18 million people are infected and about 100-120 million people are at risk of contracting the disease [61, 62].

T. Cruzi is commonly transmitted to humans by the blood-sucking triatomine bug (also known as the kissing bug) [62]. The disease is characterized by two phases: the acute infection, which occurs a few days to some weeks after the transmission, which is usually asymptomatic [62, 63]. Sometimes patients may manifest acute symptoms such as fever, inflammation at the inoculation site, unilateral palpebral edema (Romaña sign), enlarged lymph nodes, and splenomegaly that resolve spontaneously within 2-4 months. Rarely acute cardiac and neurological involvement, such as severe myocarditis and meningo-encephalitis, may also develop [64, 65].

In the absence of successful treatment, individual remains infected for life: this is the beginning of the chronic phase. Individuals may remain asymptomatic for a long period of time, usually decades, or even for their

entire life, although representing infected reservoir. Following a period of 20-30 years, approximately 30-40% of subjects develop evidence of cardiac involvement and 5-10% develop gastrointestinal signs associated with megaesophagus and/or megacolon [62, 65].

4.2.3 Chagas cardiomyopathy

Clinical involvement in Chagas disease is characterized by myocardial damage, with variable combination of LV dilatation and dysfunction, potentially leading to heart failure [62, 66-68]. The patological mechanism responsible for the adverse cardiac remodeling and chamber dilatation is represented by chronic fibrosing myocarditis, leading to progressive loss of cardiomyocites, muscle fibers and fascicles breakdown, and increasing extent of replacement myocardial fibrosis. Such profound morphological overturn is accompanied by functional alteration with decreased systolic function over time and a high likelihood of HF development [62, 67].

Furthermore, malfunctioning of the electrophysiological syncytia are responsible for potentially lethal ventricular and supraventricular tachy- and bradyarrhythmias, which can explain the high risk of sudden death in patients with CD cardiomyopathy [66].

From a physiopathological standpoint, the pathogenesis of Chagas cardiomyopathy may result from a direct tissue damage by the parasite or an indirect damage mediated by inflammatory and immune system responses [69]. Although obvious triggers of reactivation may be absent, conditions such as immunosuppression or chronic steroid treatment may predispose to chronic CD complications.

The clinical spectrum of CD cardiomyopathy may be very heterogeneous, encompassing adverse LV remodeling with chamber enlargement and systolic dysfunction or sub-epicardial fibrosis localized to the apical region.

Characteristic electrocardiographic abnormalities include right bundle-branch block (with or without associated left anterior hemiblock), premature ventricular beats, second or third degree atrioventricular block, sinus bradycardia and atrial fibrillation [68, 70]. Arrhythmias are also frequent features of CD cardiomyopathy, and may be present in the absence of detectable structural heart disease. Of note, both patients underscore the considerable arrhythmic potential of Chagas cardiomyopathy, emphasizing that CD should be considered in the differential diagnosis of “idiopathic” ventricular arrhythmias. Sudden cardiac death is common and may occur even in patients with normal ECGs [70]. A risk score for death related to CD has been developed based on 6 independent predictors, of which 3 relate to cardiovascular status, including an abnormal echocardiogram, a small QRS on the ECG and runs nonsustained VT on ambulatory Holter monitoring [68].

4.2.4 Management of Chagas cardiomyopathy

Two anti-trypansomal drugs have been introduced in the 1970s, benznidazole and nifurtimox and, although evidence from randomized control trials are lacking, benznidazole is currently considered first line therapy [71, 72]. However, while the drug is considered highly effective for eradication during the acute and congenital infection, the efficacy of treatment during the chronic phase is highly debated, where only 40-60% of patients achieving complete eradication [72]. Furthermore, treatment is associated with considerable side effects, that more frequent and

severe in adult than in children [74]. The most frequent side effects are gastrointestinal disorder, skin rash and paresthesia.

Nevertheless, because persistence of Trypanosoma Cruzi in human tissue is considered crucial for organ damage progression, antiparasitic therapy is recommended for chronic CD, especially in patients with early stages of cardiomyopathy [75].

Despite the lacking of evidence-based studies on Chagas cardiomyopathy, the specific management relies on the management of heart failure, arrhythmias and thromboembolism. Standard heart failure therapy is employed for treatment of LV dysfunction and related symptoms, including ACE-inhibitors or angiotensin receptor blockers, betablockers, anti-aldosterone agents and loop diuretics [76, 77]. Considering the high risk of thromboembolism and the elevated incidence of ischemic stroke, oral anticoagulation represent a possible important therapeutic strategy, who should guided by standard clinical recommendations.

Amiodarone is considered the agent of for treatment of ventricular arrhythmias in these patients, due to its efficacy in reducing the arrhythmic frequency, safety profile, and a reported direct antiparasitic effect [78]. Indeed, preliminary data suggest that amiodarone treatment may reduce mortality in CD patients. Selected high-risk patients with Chagas cardiomyopathy may require an implantable cardioverter-defibrillator, particularly in the presence of sustained ventricular tachycardia, syncopal episodes or persistent LV dysfunction [79].

4.2.5. Chagas disease: why should European cardiologists care?

Due to the increasing immigration, international travel and adoption, Chagas disease now represent a matter of international concern even for non-endemic countries, such as Europe or US. According to the World Health Organization, 59.000 to 108.000 individuals in Europe are affected with CD. A recent serological survey for T. cruzi infection offered to all migrants from Latin American living in the North of Italy, has shown that 4% are infected. Bolivian and Brazil migrants are at particularly high risk, with a prevalence of T. cruzi infection as high as 19.8% [58, 59].

In non-endemic countries, potential mechanism of infection include blood transfusions, transplacenteal transmission, solid organ transplantation and ingestion food or liquid contamined with feces of triatomines [62].

While screening programs of blood donors and pregnant women coming from or exposed in endemic countries are being increasingly promoted in non-endemic areas, the majority of cases are still undiagnosed or probably misdiagnosed [60]. Of note, in Europe the index of underdiagnosis of T. cruzi infection has been estimated between 94% and 96%. The long latency period from infection to the clinical manifestations, often ranging from 10 to 30 years, makes CD an extremely challenging diagnosis in non-endemic countries, where lack of awareness is a critical cause of delay or misdiagnosis. Furthermore, because of easier access to health-care compared to endemic countries, cardiac involvement in Europe and the US may be detected at the initial stages, when clinical manifestations of CD are non-specific and more difficult to interpret.

4.2.6 Conclusions

Despite its growing epidemiological relevance in the western world, and considerable emphasis by the World Health Organization, awareness of CD among physicians in Europe remains low, promoting neglect and misdiagnosis. It is hoped that CD may increasingly be considered as a potential diagnosis in European residents from endemic Latin American regions presenting with “idiopathic” cardiac manifestations, even when long removed from their country of origin, with potential implications for implementation of early treatment and control of CD transmission.

5. Translational Routes from Altered Molecular Homeostasis to Treatment Opportunities

"The art of medicine consists

in amusing the patient

while nature cures the disease"

Voltaire

Despite the modern recognition cardiomyopathies as distinct clinical entities for over five decades, and in spite of considerable advances in its phenotyping and molecular genetics, current pharmacological treatment of these patients has remained largely unchanged and empiric. In conjunction with the implementation of a DNA-based diagnosis and the comprehension of molecular phenotype, pharmacological treatment of cardiomyopathies is also likely to evolve and become largely individualized.

The overview of molecular, genetic and clinical features of cardiomyopathy reviewed in the previous sections gives the opportunity to describe the possibility to translate the acquired knowledge into treatment opportunities.

The present section illustrates novel therapeutic approaches that are being developed for patients with hypertrophic cardiomyopathy and Anderson-Fabry disease, and a critical reappraisal of a well-established – but not perfect – preventive option such as the implantable cardioverter defibrillator.

5.1 Electrophysiological cardiomyocyte remodeling as a new therapeutic target in HCM

Hypertrophic cardiomyopathy (HCM) is the most prevalent monogenic cardiac disorder, with a reported prevalence of 1 in 500 worldwide [1]. Despite its epidemiological relevance, HCM is largely an orphan condition because it lacks a disease-specific pharmacological treatment. HCM is frequently associated with reduced exercise capacity and symptoms such as angina and syncope [1].

The pathophysiology of symptoms and disease progression in HCM is complex and only incompletely understood [2-4]. However, several important elements have been identified which are closely interwoven, and impact decisively on the genesis of symptoms and long-term clinical course: A) diastolic dysfunction, multifactorial in origin, with causes ranging from intracellular calcium overload to increased interstitial fibrosis [1-4]; B) dynamic LV outflow obstruction, whether in resting conditions or during exercise [5]; C) microvascular dysfunction and blunted coronary flow reserve, secondary to marked anatomic remodelling of the arterioles [6, 7]; D) arrhythmias, with particular regard to atrial fibrillation and its consequences [8]. Moreover, HCM represents the most common cause of arrhythmic sudden cardiac death in young athletes, because of the increased propensity for potentially life-threatening ventricular arrhythmias [1]. HCM is associated with a complex electrophysiological cardiomyocyte remodelling involving multiple changes in transmembrane currents ultimately leading to susceptibility to ventricular arrhythmias; particularly, in animal models of ventricular hypertrophy, ventricular myocytes show functional down regulation of potassium currents and up-regulation of inward currents (Calcium

currents, Chloride currents, others), all leading to action potential prolongation. Prolonged action potential can lead to increased duration of QT interval at the surface electrocardiogram, a feature that has been found associated with HCM and secondary hypertrophy in patients [9-12].Marked prolongation of the QT interval is a pro-arrhythmic risk factor in both congenital and drug-induced long QT syndrome (LQTS) [11-12].

Developed in the 90s as a cardiac specific partial fatty acid oxidation inhibitor, Ranolazine was first experimented as a possible antianginal compound due to its ability to shift cardiac metabolism from fatty acid to glucose consumption, that requires less O2 to produce ATP [13]: this metabolic modulatory effect of ranolazine was thought to be responsible for its clear protective effect on myocardial tissue subjected to ischemia/reperfusion damage. It becomes clear that the antianginal and antiischemic effect of ranolazione were not due to the supposed metabolic effects (which are negligible at therapeutic concentrations), but rather to its selective blockade of the late sodium current. Its electrophysiological effects were in fact described in 2004 [14]. Ranolazine exerts an inhibitory effect on IKr and a slight ICaL block but the main effect was a potent inhibition of INaL ,with small or no effect on peak INa, within the therapeutic concentration range. Inhibition of IKr is generally considered pro-arrhythmic: however, in a large scale trial on non ST-elevation myocardial infarction patients, Ranolazine significantly reduced the incidence of ventricular tachycardia and atrial arrhythmias after the first event [15]. The late sodium current is enhanced under several pathological conditions such as ischemia, heart failure, cardiac hypertrophy and various conditions associated with oxidative stress. An increase in INaL causes Na+ overload, which in turn, promotes an increased exchange of intracellular Na+ for extracellular Ca2+ through the reverse mode of the NCX. In its reverse mode, the NCX increases Ca2+

entry into the cell in exchange to the extrusion of 3 Na+ per cycle, reducing the overall cellular capacity to eliminate calcium outside of the cytosol, and thus causing Ca 2+ overload. The antianginal effect observed at therapeutic concentrations of ranolazine (≤ 10 µM) has been related to its ability to inhibit the late INa and, as a consequence, the increase in intracellular Na+ and Ca2+ overload in myocardial cells [16].

Recently, a paper published by our group described the electrophysiological profile, Ca2+i handling properties, and contractile function of isolated cardiomyocytes and trabeculae from HCM patients undergoing surgical myectomy []. Among the several ion channel and Ca2+i handling proteins changes identified, an enhanced INaL seems to be a major contributor to the electrophysiological and Ca2+i dynamic abnormalities of ventricular myocytes and trabeculae from patients with HCM. These data suggest a favourable electrophysiological effect of ranolazine on HCM cardiomyocytes, by reverting the abnormal prolongation of action potential observed [Figure 5.1-1].

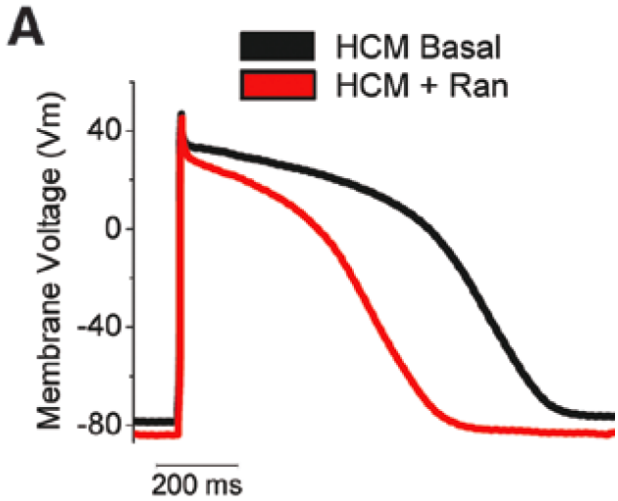


Figure 5.1-1: Effects of INaL inhibition by ranolazine on action potentials. Action potentials at 0.2 Hz from a hypertrophic cardiomyopathy (HCM) cardiomyocyte before (basal) and after exposure to 10 µmol/L ranolazine (Ran).

In HCM patients, ranolazine represents a promising option, because of its pharmacological profile, comprising several different effects of potential benefit to their condition. By reducing intracellular Na+ overload, and thus diastolic Ca2+ ranolazine has the potential to improve diastolic function, a cause of symptoms in most HCM patients, thus impacting on functional limitation and quality of life. In the long term, the effect on calcium homeostasis is expected to down regulate a number of remodelling pathways that strictly depend on myocyte driven signaling: this may in turn bring benefit to extracellular matrix remodelling, reducing fibroblasts growth and collagen production in hypertrophied myocardium. Second, ranolazine administration has been shown to reduce the degree of myocyte hypertrophy and interstitial fibrosis, and to ultimately lower the propensity to arrhythmias [18]. Third, ranolazine has been shown improve myocardial perfusion indirectly, by normalizing capillary density in the ventricles, as well as, in patients with stable CAD, via a direct action on the microvasculature leading to improved endothelial function [19]. Thus, treatment with ranolazine may be capable of alleviating angina in HCM patients, and, most importantly, to prevent disease progression towards the end-stage phase, by acting upon one of its most critical determinants - microvascular ischemia. In principle, ranolazine has an ideal profile for clinical use in HCM patients, suggesting a wide range of positive actions which may critically impact on acute symptoms as well as on the natural history of the disease. Such a strong rationale calls for a concerted action aimed at investigated the efficacy of ranolazine in this not-so-uncommon disease, which is still orphan of appropriate pharmacological treatment.

In the light of this view, a pilot study has been designed in order to test the effectiveness of ranolazine on exercise tolerance and symptoms in HCM patients. The present study is intended to represent the first

meaningful step in such direction, by assessing the clinical and instrumental effects of a 5-month treatment with ranolazine in a selected cohort of HCM patients.

The protocol of this randomized, Double blind, Placebo controlled, study is here presented: a total of 120 patients with symptomatic HCM (SHCM) will be recruited in 11 European referral centers and randomized to receive ranolazine or placebo for 5 months. During the 5 months treatment phase, the randomized patients will perform 3 visits, for safety and efficacy assessments [Figure 5.1-2].

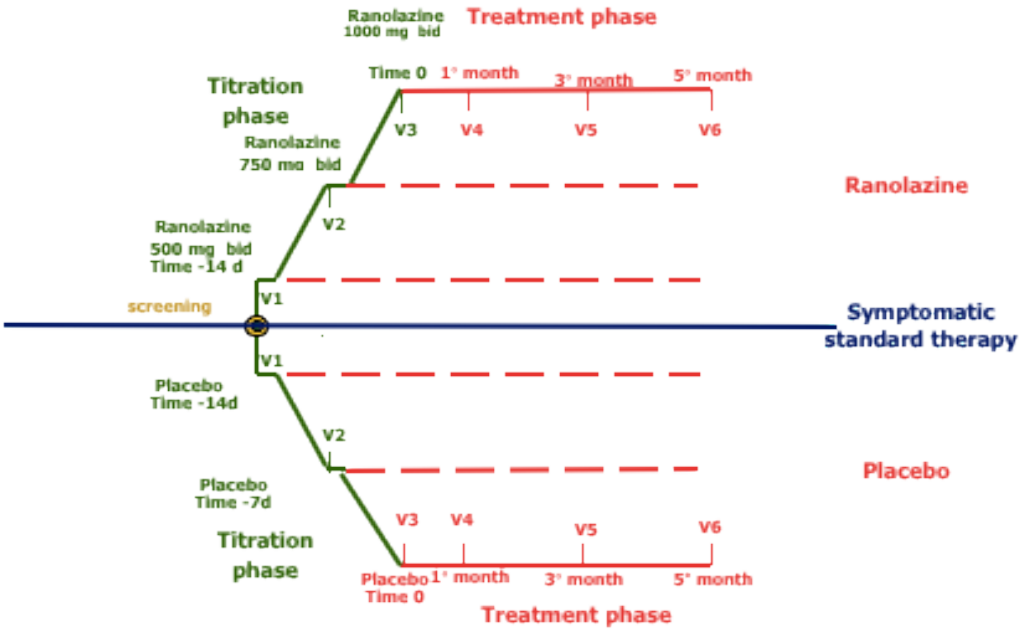


Figure 5.1-2: study design.

Visit 1 to visit 2: up-titration of drug dose.

Visit 4 to Visit 6: treatment period

Study name and code:

RESTYLE-HCM: RANOLAZINE IN PATIENTS WITH SYMPTOMATIC HYPERTROPHIC CARDIOMYOPATHY: A PILOT STUDY ASSESSING THE EFFECTS ON EXERCISE CAPACITY, DIASTOLIC FUNCTION AND SYMPTOMATIC STATUS

Code: MEIN/11/RAN-HCM/001 (EUDRA-CT number: 2011-004507-20)

Improvement in exercise capacity, as assessed by cardiopulmonary test with the Peak Oxygen Consumption (V02 peak) technique, will be the primary endpoint.

Secondary objectives will be to demonstrate the efficacy of ranolazine on diastolic function, symptomatic status and natriuretic peptide biomarker proBNP in patients affected by SHCM. The assessment of safety by evaluation of adverse events, laboratory findings, the rest standard 12-lead ECG, detection of 24-hour arrhythmic burden by Holter ECG monitoring and physical examination will also be considered a secondary study objectives.

The ongoing study is still recruiting patients in Europe, with the leading center of Florence with the highest number of enrolled individuals. Despite the idea of using established drug therapies in an “old” disease such HCM, the real purpose of the study is to take a step toward the world of evidence-based medicine.

The development of new experimental drugs, such new sodium channel blocker, more specific than ranolazine, is ongoing. Interest of pharmaceutical company in this fascinating group of disease is growing faster and the possibility to intervene on the complex pathophysiology of the disease in order to alter its natural course is close at hand.

5.2 The real story of life-saving devices: a backstage tour

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease, with mutations in genes coding for myofilament contractile proteins of the cardiac sarcomere representing the most common genetic substrate. In most cohort studies the prevalence of genotype positive patients is as high as 65%, representing over two third of the total [20]. To date, large clinical studies on HCM have assessed outcome irrespective of genetic background. Although clinically indistinguishable, this genotype-negative subset is likely to include a number of rare etiologies, including phenocopies, like infiltrative disease, whose natural history might differ significantly from sarcomere-related HCM [20, 21].

Therefore, the large proportion of patients with no detectable sarcomere myofilament gene mutations may confound the natural history of HCM with proven sarcomere etiology. Furthermore, such concept is supported by the finding that genotype-positive patients have more severe outcome compared to those who are genotype-negative [20]. Thus, data deriving from non-genotyped populations may significantly confound our perception of the clinical correlates and outcome associated with a specific genetic background, and large studies on genotyped HCM populations appear desirable [22].

In order to address this important issue, we sought to investigate the clinical features and outcome of a large cohort consisting solely of patients with proven myofilament-positive HCM, followed prospectively at our Institution after systematic genetic screening. In addition, the impact of the implantable cardioverter-defibrillator (ICD), both in terms of

appropriate intervention rates and adverse effects, were specifically assessed.

5.2.1 Clinical picture of the study cohort

We followed for 6±3 years 250 HCM patients (age 46±17 years, 36% female) consecutively found positive at mutational screening in the protein-coding exons and splice sites of 8 sarcomere gene, including *myosin binding protein C* (MYBPC3), *beta-myosin heavy chain* [MYH7], *regulatory and essential light chains* [MYL2 and MYL3], *troponin-T* [TNNT2], *troponin-I* [TNNI3], *alpha-tropomyosin* [TPM1], and *alpha-actin* [ACTC]).

The distribution of affected genes in this population was consistent with most reported cohorts internationally, with a large majority carrying single mutations in either of MYBPC3 and MHY7 gene [Figure 5.2-1]. Only 20 patients (8%) had complex genotypes characterized by two or more mutations in the same or in different sarcomere genes.

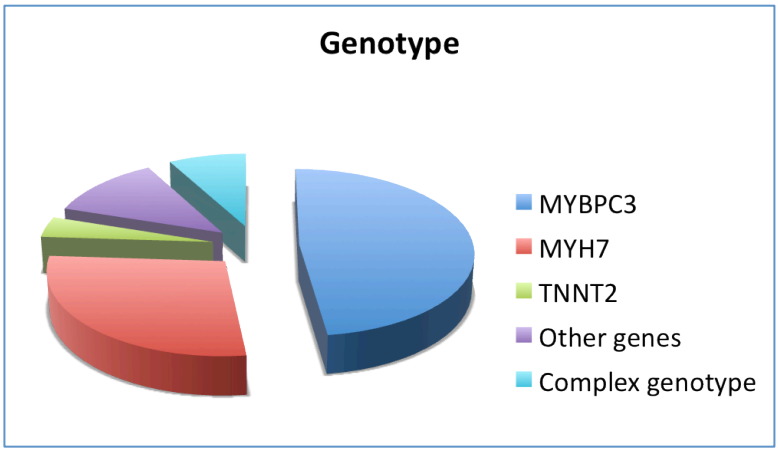


Figure 5.2-21: Distribution of sarcomere mutation in the study cohort

MYBPC3 (48%), MHY7 (28%), TNNT2 (4%), or the other genes (12%); complex genotype (8%).

Overall, 64 patients (26%) received an ICD, for secondary (n=10) or primary prevention of SCD (n=54). Among the latter, the ICD was implanted because of the high-risk profile (n=37) or progression to overt LV systolic dysfunction (LVEF<50%; n=17, including 10 with CRT capabilities) [Figure 5.2-2]. Three clinical scenarios led to the recommendation of ICD implantation in individual our HCM patients: 1) prior cardiac arrest or sustained ventricular tachycardia (secondary prevention); 2) primary prophylaxis of SCD in patients with ≥2 of the established risk factors indicated by current guidelines, i.e. malignant family history, extreme LV wall thickness, unexplained syncope, abnormal blood pressure response to exercise and repeated runs on nonsustained ventricular tachycardia (NSVT) on 24-hour ECG monitoring; 3) progression towards systolic dysfunction, the so-called "end-stage" phase, per se associated with high SCD rated, in whom cardiac resynchronization was routinely considered at the time of ICD implantation.

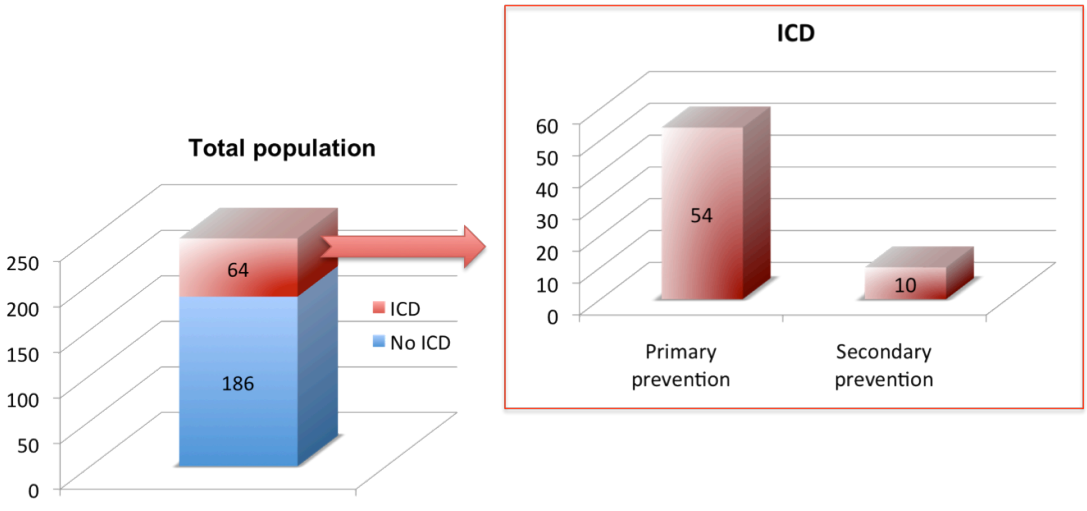


Figure 5.2-2: Patients with ICD

64 patients had an ICD implanted for primary or secondary prevention (respectively 54 and 10).

5.2.2. Outcome

At final evaluation, the incidence of death from cardiovascular causes was quite low, with an overall 6% and an annual incidence of 1%. Indeed, the most frequent cause of death was related to progressive heart failure, while SCD appeared to be less frequent.

The comparison of subgroups of patients, based on affected gene was not statistically significant, although complex genotypes showed more severe clinical course and adverse outcome. However, while the incidence of SCD and appropriate ICD discharge were comparable to previously reported unselected HCM cohorts, progression to overt systolic dysfunction was more common in our cohort of genotype-positive patients. Thus, the significant propensity towards LV systolic dysfunction and HF, with a prevalence of 10 and 14% respectively, is consistent with the hypothesis of long-term energy depletion due to inefficient energetic handling by the mutated sarcomere. Indeed, this concept is accentuated in patients with multiple genetic defects, representing extreme paradigms of deranged sarcomere function.

Compared to patients without ICD, those who received a device were younger at HCM diagnosis, more symptomatic, had greater maximum LV wall thickness and left atrial dimensions, and, as expected, a greater number of risk factors for SCD. Thus, patients with ICD had an unfavorable baseline risk profile, with a greater prevalence of end-stage disease, which is known to be associated with a particularly ominous outcome. Despite that, the incidence of cardiovascular mortality was only slightly higher, but not statistically significant, from that observed in patients without ICD [Figure 5.2-3]. Of note, two of the deaths in the ICD group were sudden, occurring despite the device: unfortunately, neither

an autopsy nor an ICD interrogation was performed in these patients, and the exact causes of death could not be ascertained.

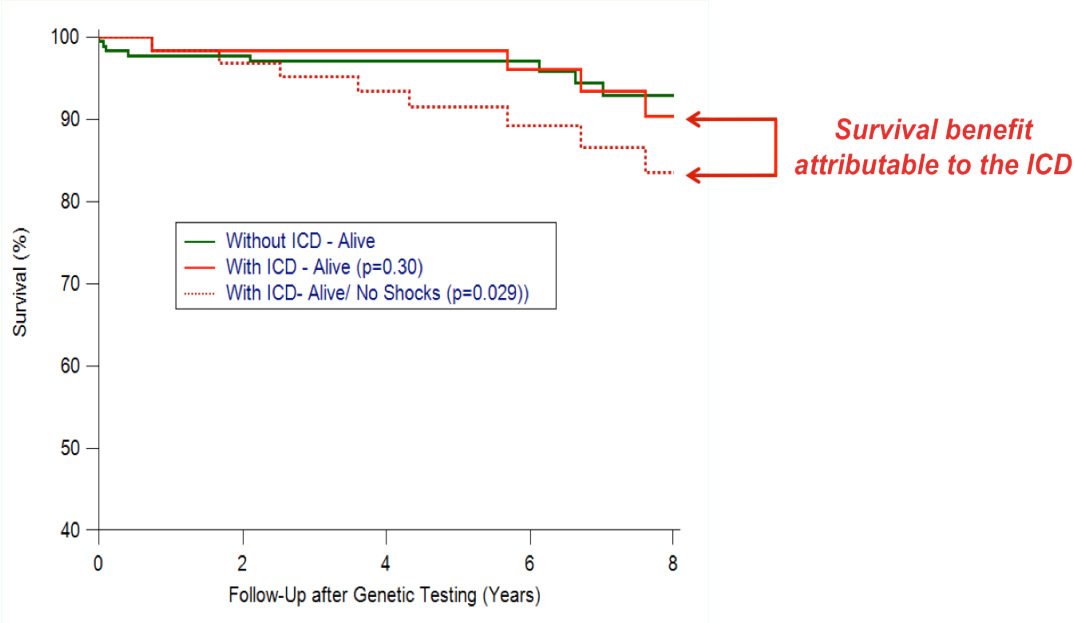


Figure 5.2-3: Survival benefit attributable to the ICD

ICD allowed favorable (or normalize) survival rates in a subset of HCM patients.

5.2.2. ICD: the good, the bad and the ugly

Implantable cardioverter defibrillators have now been in clinical practice for 25 years. Despite great skepticism and opposition in the early years, the weight of data from multiple prospective randomized clinical trials has proved beyond any doubt that ICD therapy is highly effective in reducing sudden cardiac death in patients with a great variety of cardiac pathologies. Faced with 'evidence-based medicine', HCM guidelines have become increasingly clear, and we all now recognize that there are patients for whom an ICD is highly recommended. Furthermore, multiple trials of ICD treatment compared with drugs showed that in specific clinical setting, ICD therapy might be superior to drugs.

Overall, 11% of patients experienced an appropriate ICD intervention (shocks for VT of VF), with an annual incidence of 2%. Therefore, ICD allowed favorable survival rates in a subset of high-risk HCM patients [Figure 5.2-3].

However, 16 patients (25% of the ICD subset, including 2 with appropriate shocks) experienced device-related complications such as inappropriate ICD interventions (n=10; including 4 with electric storms due to lead fracture), infections (n=4) and lead dislocation (n=6) [Figure 5.2-4]. As a consequence of multiple inappropriate shock and psychological distress, two young patients required their ICD to be explanted, 5 and 7 years after implantation; both are still alive. Finally, 31 patients required one or more substitutions of the device at end of battery life.

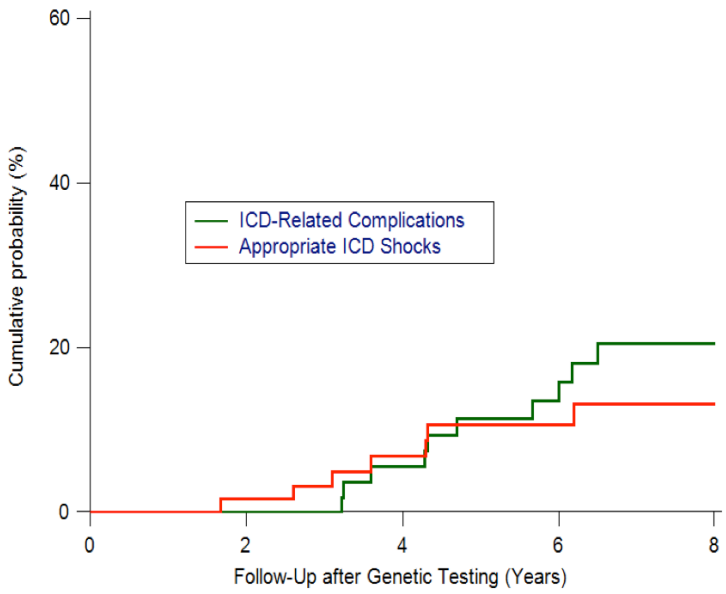


Figure 5.2-4: ICD-related complications

Overall, 16% of patients with ICD experienced device-related complication, while only 11% had appropriate device intervention. The rate of complication increase over time and become significant after 3 year from implantation.

Therefore, the decision to implant an ICD prophylactically for SD prevention in HCM patients involve consideration of the potential complications and inconvenience incurred by a permanent device versus obvious lifesaving benefit [23, 24]. Particularly, these considerations should arise in pediatrics patients when physicians are confronted by the clinical paradox in which active and healthy-appearing HCM patients (exposed to greatest SD risk by age) have the highest device complication rates over long time periods [25]. Device-related complications, including infection, pocket hematoma, pneumothorax, and venous thrombosis, are well documented and occur most commonly in younger patients, primarily because their activity level and body growth place continual strain on leads, considered the weakest link in this system [25-27].

Although controversial, there is evidence that defibrillator shocks can cause myocardial damage [28, 29], and the shocks have been associated with increased mortality [30]. Recent findings from one large randomized trial showed that different programming approach may reduce potentially dangerous inappropriate therapies and increased survival among patients with ICDs [31]. Furthermore, device technologies move on at a fast rate and new alternative to the classic intravenous ICD are currently available, although with limited clinical experience especially in patients with cardiomyopathy [32].

5.2.3 Conclusions

In our large cohort of HCM patients with proven sarcomere myofilament disease, risk of sudden cardiac death or appropriate ICD shock was low even in the presence of multiple risk factors. Conversely, end-stage progression proved relatively common, supporting the hypothesis of long-term myocyte energy depletion related to mutations in sarcomeric genes.

The ICD allowed favorable survival rates in a subset of HCM patients at higher risk, at the cost of considerable complication rates, and sudden cardiac death occasionally occurred despite the device.

5.4 Changing the destiny of unfolded proteins: pharmacological chaperone as a new therapy for Anderson-Fabry disease

Fabry disease is a hereditary, X-linked lysosomal storage disorder caused by a deficiency of the lysosomal enzyme α-galactosidase A, which results in the accumulation of the glycosphingolipid globotriaosylceramide (Gb3), in different cells and organs, especially in endothelial cells and smooth muscle cells of blood vessel [33, 34].

To date, more than 500 mutations have been reported for the GLA gene, 57% of which are missense mutations. Patients with missense mutations often have some residual enzyme activity, ranging from 2% to 25% [33]. Studies of the residual GLA activity of mutant forms of the enzyme revealed that many had kinetic properties similar to the wildtype enzyme, but were significantly less stable [35-37]. These results suggest that the compromised enzyme activity in many Fabry patients is due to protein misfolding and the inability to traffic the enzyme to the lysosomes, where they are needed to break down substrate [36]. Misfolded enzymes may be stopped by the ER quality control system and the mutant protein is retained in the ER and degraded prematurely [Figure 5.4-1]. Since most misfolded enzymes are never sent to the lysosome, substrate may accumulate within the lysosome, damage the cell, and cause the signs and symptoms of a lysosomal storage disorder [36]. Therefore, the discovery of small molecules that assist mutant enzymes to fold correctly may rescue them from premature degradation and increase the amount of active enzyme in the lysosomes.

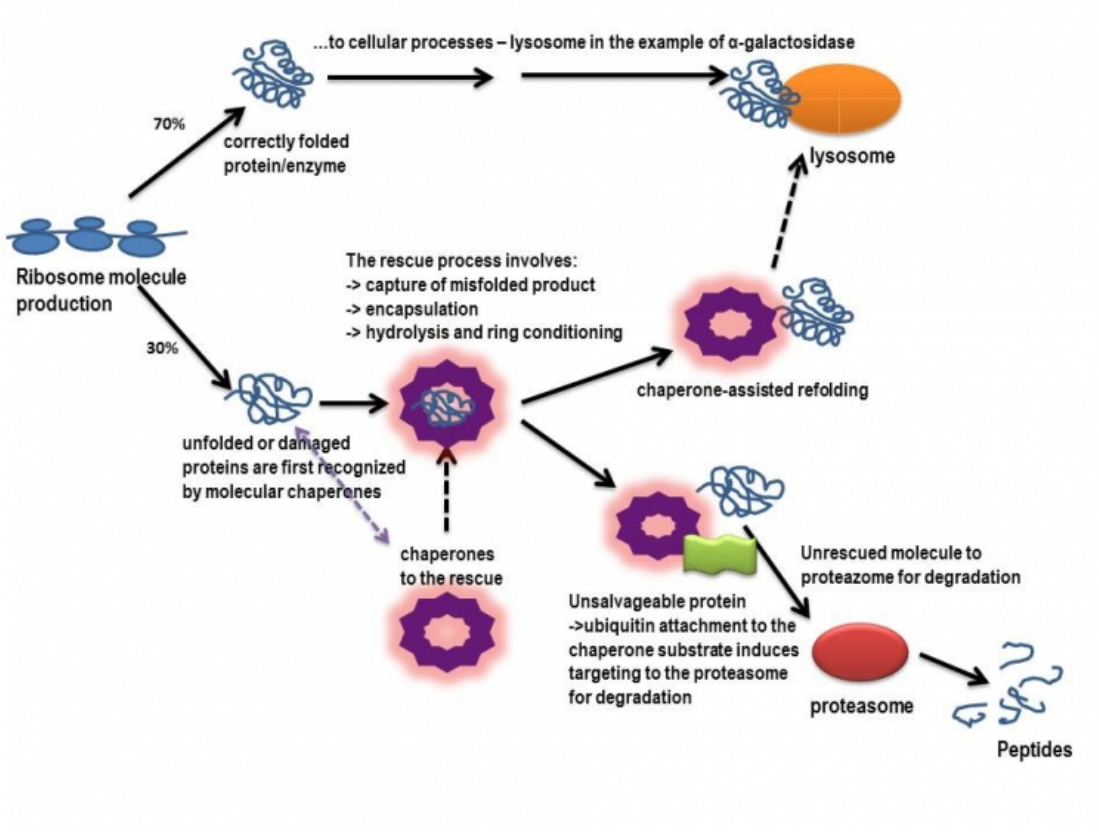


Figure 5.4-1: A simplified schematics of the molecular chaperones rescue mechanism, sorting proteins for folding or degradation

ERT is presently considered the standard of care for LSDs [38]. It has considerable success in the treatment or in ameliorating the quality of life of patients with several of these diseases. However, ERT has significant limitations in terms of tissue distribution of therapeutic enzymes, impact on patients' quality of life and high costs []. ERT is based on the intravenous infusion of recombinant or gene-activated human enzyme. The enzyme is delivered into the blood in order to reach specific receptor cell surfaces, to be taken up by cells and then transported to the lysosome. Upon entering the lysosome, this enzyme can perform the function of the unstable endogenous enzyme. However, the pH in the infusion bag and in blood is higher than the enzyme's natural acidic

environment in the lysosome. In result, the infused enzyme rapidly denatures and may be misdirected to non-target tissues or delivered in inactive form to the lysosome. Exposure to these infused enzymes may also mount an immune response or neutralizing antibodies than can impact efficacy or cause adverse effects. Possible problems related to the unfolding of infused enzyme include: rapid denaturation and misfolding in blood, leading to short circulating half-life, immunogenicity, poor delivery and uptake of active enzyme into key tissues of disease and reduced activity.

Pharmacological chaperone therapy (PC) has recently emerged as a potential therapeutic alternative for Fabry disease and appears advantageous when compared to ERT, as the chaperones are better distributed in tissues, including the brain, and because therapy may be administered orally [36, 37, 40]. PCT, however, also has limitations, since only patients with responsive mutations will be amenable to this therapy [40]. Pharmacological chaperones are small molecules that are designed to selectively bind to a misfolded protein thereby increase the protein's stability, assisting their correct folding, maturation, and trafficking to their functional site, such as the lysosome [Figure 5.4-1 and 5.4-2]. Once in the lysosome, the pharmacological chaperone disassociates and the enzyme is free to break down substrate []. By stabilizing a misfolded protein, pharmacological chaperones may be able to restore the intended biological function of the protein. Because each chaperone is designed to bind to only one particular lysosomal enzyme, a specific chaperone is developed for each targeted lysosomal disease []. PC are reversible enzyme inhibitors and bind to the active enzyme site, thus stabilizing, but also inhibiting, the enzyme. To be effective, PC must be able to dissociate from the enzyme once they have reached the lysosome, a process which is promoted by the acidic environment of lysosomes and excess of natural

substrate, with a higher affinity to the catalytic sites []. Therefore, the concentration of chaperone in the ER and in the lysosome is a critical factor. If the amount of chaperone in the ER is low, they are ineffective in assisting the enzyme folding, thus resulting in a reduced enzyme concentration (and activity) in the lysosome. However, the dissociation of the PC from the active site of the enzyme is impossible if the concentration of chaperone in the lysosome is too high. In that case, the PC occupies the active site of the enzyme and the enzyme activity is therefore reduced. In theory, an enzyme activator or a pure enzyme binder with chaperone activity would be a better choice as a candidate for drug development. If an enzyme activator is used as a therapy, both the chaperone and enzyme stimulatory actions of the activator would synergistically increase enzyme activity in the lysosomes.

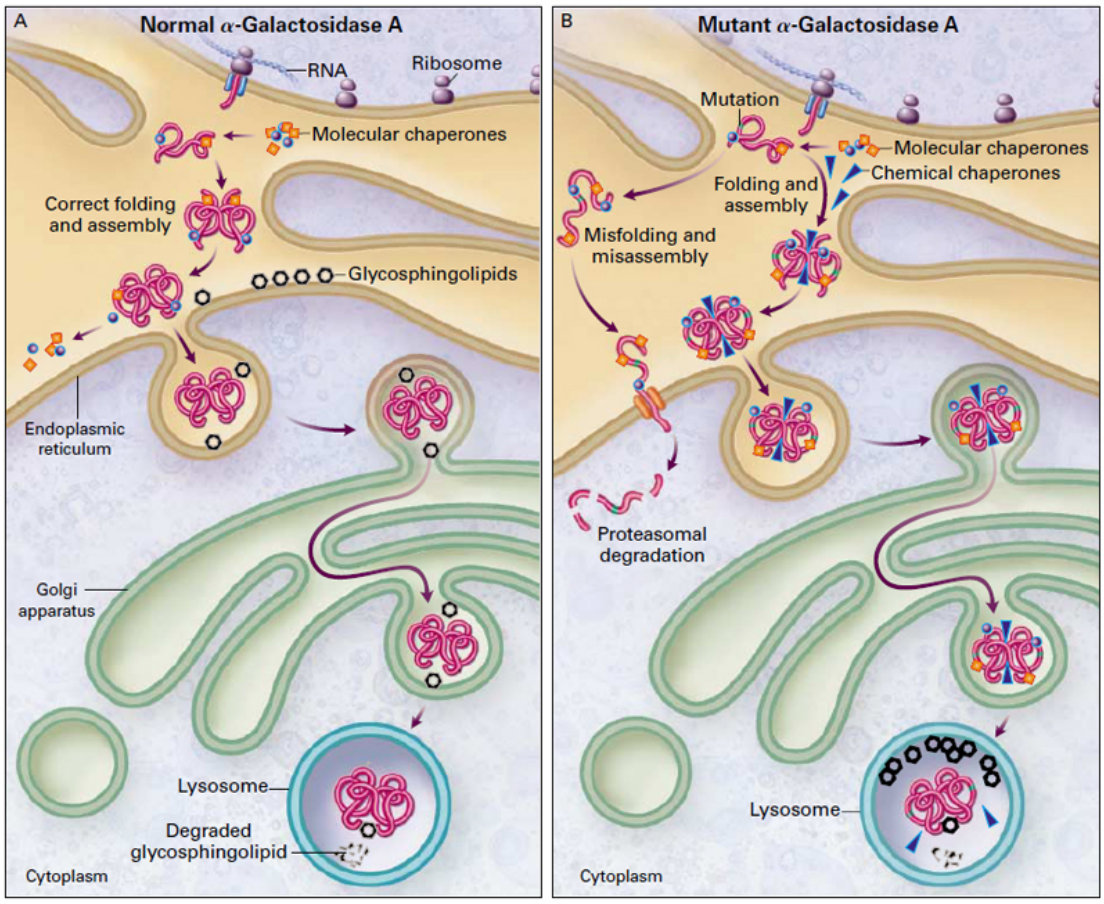


Figure 5.4-2: Proposed Mechanism of Enzyme Stabilization by Chemical Chaperones.

Panel A shows the processing of normal a-galactosidase A. Newly synthesized a-galactosidase A is translocated into the endoplasmic reticulum, where molecular chaperones facilitate its proper folding and dimerization by specialized processing enzymes. The molecular chaperones then dissociate from the folded, dimerized enzyme, which moves to the Golgi apparatus and then to lysosomes, where the enzyme is stable and active in the acidic environment of these organelles. Panel B shows the processing of mutant a-galactosidase A. Most mutations in the α-galactosidase A gene encode α-galactosidase A molecules that are misfolded, misassembled, or aggregated in the endoplasmic reticulum, where they are degraded, presumably by the ubiquitin–proteasome pathway. However, certain missense mutations decrease the stability of the enzyme, but the conformation of the active site is retained. Most of this type of mutant enzyme is degraded in the endoplasmic reticulum. However, these mutant forms of α-galactosidase A may be stabilized by chemical chaperones, such as galactose, that bind to the active site of the enzyme, promote folding, and stabilize the mutant enzyme. Some of the enzyme then reaches the lysosomes, where it retains low levels of activity. In the lysosomes, the accumulated glycosphingolipid substrates displace the chemical chaperones and are hydrolyzed by the enzyme [Modified from 44].

Recent studies suggest that new solutions to improve the efficacy of treatments for LSDs may lie on the combination of distinct therapies [45]. The combination of ERT and PCT resulted in a synergistic effect in Pompe and Fabry disease models, and may be helpful in patients responding poorly to ERT and in tissues where corrective levels of recombinant enzymes are difficult to obtain. These studies greatly expand the use of pharmacological chaperones and suggest a change in the use of these drugs. One GLA inhibitor, 1- deoxygalactonojirimycin (DGJ, marketed as AmigalTM by Amicus Therapeutics, Inc. [46]) is currently being studied, as a chaperone therapeutic agent for Fabry disease, in phase 2 and phase 3 clinical trials:

1) Migalastat HCl Monotherapy: Phase 3 Study 011 (The FACETS, or FAB-AT1001-011 Study): Study of the Effects of Oral AT1001 (Migalastat Hydrochloride) in Patients with Fabry Disease. Design: Placebo-controlled, double-blind Phase 3 study of migalastat HCl

2) Migalastat HCl Monotherapy: Phase 3 Study 012 (The ATTRACT, or FAB-AT1001-012 Study): Study to Compare the Efficacy and Safety of Oral AT1001 and Enzyme Replacement Therapy in Patients with Fabry Disease. Design: Randomized, open-label, 18-month Phase 3 study investigating the safety and efficacy of migalastat HCl compared to current standard-of-care ERTs Fabrazyme® (agalsidase beta) or Replagal® (agalsidase alfa) for Fabry disease

3) Migalastat HCl Monotherapy: Phase 2 Extension Study 205 (FAB-CL-205 Study): Open Label Long-term Safety Study of AT1001 in Patients with Fabry Disease Who Have Completed a Previous AT1001 Study. Design: Open-label Phase 2 extension study to evaluate the long-term safety and tolerability and to explore the efficacy of migalastat HCl in patients who have previously completed a Phase 2 clinical study of migalastat HCl for Fabry disease.

4) Migalastat HCl Co-Administered with ERT: Phase 2 Study 013 (AT1001-013 Study): Drug-Drug Interaction Study Between AT1001 and Agalsidase in Subjects With Fabry Disease. Design: Open-label Phase 2 study to compare a single administration of oral migalastat HCl co-administered with infused ERT (Fabrazyme or Replagal) versus ERT alone. Each patient receives their current dose and regimen of ERT alone at one infusion and oral migalastat HCl (150 mg or 450 mg) administered prior to ERT at their next infusion

5.2.1 The ATTRACT study

ATTRACT is an acronyms for "**AT**1001 **t**herapy compared to enzyme **r**eplacement therapy in Fabry patients with **A**T1001-responsive mutations: a global **c**linical **t**rial". The ATTRACT study is a phase 3 clinical trial that aims to measure the effectiveness and safety of an investigational pharmacological chaperone migalastat HCl (AT1001), when compared to enzyme replacement therapy (ERT) alone, for the treatment of patients with Fabry disease.

The study enrolled approximately 50 participants currently receiving agalsidase (ERT), who have been randomized to receive either migalastat HCl or agalsidase (ERT). Approximately 30 participants stopped their agalsidase (ERT) regimen and started with migalastat HCl and 20 participants remained on their prescribed agalsidase (ERT) regimen. The ATTRACT study was fully enrolled on December 4th, 2012.

Study participation will last approximately 21 months, including a screening/baseline period (2 months), an open-label treatment period (18 months), and a follow-up period (1 month). After completing the 18 month treatment period, participants will have the option to continue in a 12-month treatment extension, where all participants will be treated with *Migalastat*.

The primary end-point of the study is the assessment of renal function by measuring the glomerular filtration rate (GFR) as assessed by plasma clearance of iohexol ("iohexol GFR"). Iohexol is a non-ionic x-ray contrast medium of low osmolality, extensively used in clinical radiology and considered essentially free from side effects. Like other iodine-containing contrast media, it is eliminated from the body by excretion in the urine. These substances are therefore potential markers for renal function. Iohexol is a suitable marker for glomerular filtration rate (GFR):

after intravenous injection, it is quantitatively recovered in the urine; the elimination occurs by glomerular filtration, with no signs of tubular secretion or reabsorbtion.

The secondary end-points are more specifically focused on those signs expression of major organ involvement, such as 24-hour urine protein for kidney disease, cardiac morphology and function assessed by echocardiography, burden of pain and quality of life reported by the patients (standard questionnaire). Furthermore, a composite clinical outcome is also assessed by the occurrence of renal, cardiac, cerebrovascular events or death.

Our center is actively involved in the ATTRACT study. Two female patients were enrolled on July 2012 and both randomized to receive *Migalastat* and stop ERT. So far, no serious adverse events have been reported in our patients.

5.2.2 Next-Generation Approach for Lysosomal Storage Diseases (LSDs)

A substantial amount of novel and promising data support the potential of pharmacological chaperones as therapeutic agents in Fabry disease [36]. Future efforts should be directed towards the identification of new chaperones for additional LSDs and the identification of 'second-generation' molecules with a good safety profile and better enhancing properties and intracellular distribution [42]. An ideal compound should have weaker inhibitory effects and higher enhancing activity. Novel chaperone molecules should not only be advantageous in terms of improved enhancing profile, but may also increase the rate of responsive mutations by assisting the folding of enzymes with mutations in domains other than the catalytic site.

Given the limitations of all therapeutic approaches so far, looking for new strategies to optimize therapies and to target the diverse cellular and phenotypic aspects of these diseases is becoming an important goal of current research. In this respect, a leap forward was made, thanks to recent studies that indicated new solutions to improve the efficacy of treatments for LSDs and opened new avenues to the use of PCT. Although the concept of PCT is based on the enhancing effect of chaperones on mutated misfolded proteins, some evidence points to a possible effect of chaperones on wild-type enzymes [45, 40]. Chaperones induce conformational stabilization and increase thermal stability of normal recombinant enzymes used for ERT, which may be prone to mistrafficking and degradation like the mutant enzymes. Some studies suggested that protection of recombinant enzymes from degradation can be achieved by chaperones [47].

The CHART™ (Chaperone-Advanced Replacement Therapy) platform combines unique pharmacological chaperones with enzyme replacement therapies (ERTs) for LSDs. Amicus is leveraging the CHART platform to improve currently marketed ERTs through co-administration of a pharmacological chaperone prior to ERT infusion, and to develop next-generation ERTs that consist of a proprietary lysosomal enzyme therapy co-formulated with a pharmacological chaperone. This platform has demonstrated proof-of-concept in the clinic and in multiple preclinical studies. In the clinic, consistent increases in active enzyme in plasma and enzyme uptake into tissue have been observed following chaperone-ERT co-administration compared to ERT alone. In preclinical studies, this increased activity and tissue uptake has lead to greater substrate reduction than ERT alone. In each CHART program, a unique pharmacological chaperone is designed to bind to a specific therapeutic (exogenous) enzyme, stabilizing the enzyme in its properly folded and

active form. This may allow for enhanced tissue uptake, greater lysosomal activity, more reduction of substrate, and lower immunogenicity compared to standard of care ERTs. This combination approach may benefit patients with lysosomal storage diseases, including patients with inactive endogenous proteins who are not amenable to chaperone monotherapy. Therefore, such coadministration may provide an improved therapeutic strategy that is broadly applicable to a diverse population of Fabry patients.

6. Conclusive Remarks

"An investment in knowledge

pays the best interest."

Benjamin Franklin

Conclusive remarks

New hopes

After more than fifty years of passionate clinical and molecular description of cardiomyopathy, the enthusiasm for these fascinating heart diseases has reached a record high level. Now, the time is ripe to consider what we know and reflect on what remains to be discovered in order to fill the existent gaps.

The interest of the scientific world and pharmaceutical companies is slowly emerging, giving to physicians and researchers involved in the fields a remarkable opportunity to translate basic insights into new clinical models for diagnosis, prevention, and therapy. Novel initiatives, supported by private companies and international medical societies, are paradigmatic examples of the increased awareness and subsequent interest in this group of diseases.

The EURObservational Research Programme (EORP) was launched by the European Society of Cardiology in 2009, with the aim to provide a better understanding of medical practice, based on observational data collected with more robust methodological procedures. Three years later, a special EORP Registry on Cardiomyopathies was added to the list, with the intent to collect previously unobtainable data on the epidemiology and outcomes of patients seen across referral centers in Europe. The ESC Cardiomyopathy Registry is a prospective, multicenter, observational study that describes the demographic, clinical and genetic characteristics of patients with cardiomyopathies. The study was conducted as a pilot for the first year in a small number of expert centers, with 1149 patients enrolled in the first twelve months, and the long-term registry will start in 2014.

The call for action, emphasized also in an official report of international working group [1], lighted up the enthusiasm for cardiomyopathies, making them a group of diseases particularly appealing for investments of private and public companies, and the number of clinical trials, as well as the development of new specific therapeutic strategies, will hopefully increase in the immediate and near future.

In conclusion, cardiomyopathies are not uncommon, but remain orphan diseases with regard to treatment. In the era of translational research, physicians and researchers have been very good at asking the right questions, but they need to direct more effort into finding the best clinical answers for these patients. The changing epidemiology of these diseases now allows what was previously impossible: it is high time to move cardiomyopathies into the era of evidence-based management.

In a broader perspective, cardiomyopathies should be seen as paradigms providing invaluable insights on disease mechanisms that may be of general relevance to patients with more prevalent cardiac conditions. What has long been considered impossible to achieve is now closer at hand: the time is now ripe to promote robust clinical research in these complex conditions, in order to advance and standardize management of patients.

7. References

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Chapter 2: Genotype-Phenotype Correlations

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Chapter 6: Conclusive remarks

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8. Publications

"If the facts don`t fit the theory,
change the facts"

Albert Einstein

8.1 List of publications

1. Tomberli B, Cecchi F, Sciagrà R, Berti V, Lisi F, Torricelli F, Morrone A, Castelli G, Yacoub MH, Olivotto I. *"Coronary microvascular dysfunction is an early feature of cardiac involvement in patients with Anderson-Fabry disease."* **Eur J Heart Fail** 2013 Dec; 15(12):1363-73. [Full text in section 8.2]
2. Olivotto I, Maron BJ, Tomberli B, Appelbaum E, Salton C, Haas TS, Gibson M, Nistri S, Servettini E, Chan RH, Udelson JE, Lesser JR, Cecchi F, Manning WJ, Maron MS. "*Obesity and its Association to Phenotype and Clinical Course in Hypertrophic Cardiomyopathy.* " **J Am Coll Cardiol** 2013 Jul 30;62(5):449-57. [Full text in section 8.2]
3. Castelli G, Fornaro A, Ciaccheri M, Dolara A, Troiani V, Tomberli B, Olivotto I, Gensini GF. "*Improving survival rates of patients with idiopathic dilated cardiomyopathy in Tuscany over three decades: impact of evidence based medicine*". **Circulation Heart Failure** 2013 Sep 1;6 (5):913-21. [Full text in section 8.2]
4. D'Amato R, Tomberli B, Castelli G, Spoladore R, Girolami F, Fornaro A, Caldini A, Torricelli F, Camici P, Gensini GF, Cecchi F, Olivotto I. "*Prognostic Value of N-Terminal Pro-Brain Natriuretic Peptide in Outpatients With Hypertrophic Cardiomyopathy*". **Am J Cardiol**. 2013 Oct 15;112 (8):1190-1196. [Full text in section 8.2]
5. Cecchi F, Tomberli B, Morrone A. "*Anderson Fabry disease, the histrionic disease: from genetic to clinical management*." **Cardiogenetics**, 2013, 3 (1s), e3. [Full text in section 8.2]
6. Olivotto I, Tomberli B, Spoladore R, Mugelli A, Cecchi F, Camici PG. "H*ypertrophic cardiomyopathy: the need for randomized trials*". **Global Cardiology Science and Practice** 2013:31. [Full text in section 8.2]
7. Tomberli B, Girolami F, Coppini R, Ferrantini C, Rossi A, Cecchi F, Olivotto I. *"Management of refractory symptoms in hypertrophic*

cardiomyopathy with restrictive pathophysiology: novel perspectives for ranolazine". [Article in Italian] **G Ital Cardiol** (Rome) 2012 Apr;13 (4):297-303. [Full text in section 8.2]

8. Cecchi F, Tomberli B, Olivotto I. "*Clinical and molecular classification of cardiomyopathies*." Review article. **Global Cardiology Science and Practice** 2012. Vol. 2012 1, 4. [Full text in section 8.2]

9. Girolami F, Bardi S, Berti L, Cecchi F, Servettini E, Tomberli B., Torricelli F, Olivotto I. "Rilevanza clinica del test genetico nella cardiomiopatia ipertrofica" [*Clinical relevance of genetic testing in hypertrophic cardiomyopathy*]. **Recenti progressi in medicina**, 2011 vol. 102; p. 486-493. [Full text in section 8.2]

10. Chan RH, Maron BJ, Olivotto I, Assenza EG, Lesser JR, Haas T, Gruner C, Crean AM, Rakowski H, Udelson JE, Rowin E, Lombardi M, Tomberli B, et al. "*Prognostic Utility of Quantitative Contrast-Enhanced Cardiovascular Magnetic Resonance in Hypertrophic Cardiomyopathy.*" **Sumbitted**. [Abstract in section 8.2]

11. Vannucchi V, Tomberli B, Zammarchi L, Fornaro A, Castelli G, Pieralli F, Berni B, Yacoub S, Bartoloni A, Olivotto I. *"Chagas disease as a cause of heart failure and ventricular arrhythmias in patients long removed from endemic areas: an emerging problem in Europe"*. **Journal of Cardiovascular Medicine**, in press. [Abstract in section 8.2]

12. Girolami F, Iascone M, Tomberli B, Bardi S. Benelli M, Marseglia G, Pescucci C, Pezzoli L, Sana ME, Basso C, Marziliano N, Merlini P.A, Fornaro A, Cecchi F, Torricelli F, Olivotto I. *"Novel alpha-actinin-2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: a massively parallel sequencing study"*. **Submitted**. [Abstract in section 8.2]

13. Coppini R, Ho CY, Ashley E, Day S, Ferrantini C, Girolami F, Tomberli B, Bardi S, Torricelli F, Cecchi F, Poggesi C, Tardiff J, Olivotto I."

Clinical phenotype and outcome of Hypertrophic Cardiomyopathy associated with sarcomere thin filament gene mutations". **Submitted**. [Abstract in section 8.2]

14. Tomberli B, Olivotto I, Ferrantini C, Coppini R, Fornaro A, Girolami F, Castelli G, Pieragnoli P, Padeletti L, Cecchi F. *"Outcome of Hypertrophic Cardiomyopathy associated with sarcomere protein gene mutations: impact of the Implantable Cardioverter-Defibrillator"*. [Abstract in section 8.2]

15. B. Tomberli, A. Fornaro, S. Bardi, F. Torricelli, M. Benelli, C. Pescucci, F. Cecchi, F. Girolami, I. Olivotto. *"A novel desmoplakin dominant mutation responsible for Carvajal/Naxos syndrome identified by exome sequencing"*. [Abstract in section 8.2]

16. E.J. Rowin, B. Tomberli, A. Arretini, B.J. Maron, S.A. Casey, R. Garberich, M.S. Link, R.H.M. Chan, E. Appelbaum, J.R. Lesser, J.E. Udelson, M.S. Maron, F. Cecchi, I. Olivotto. *"Prevalence and clinical significance of heart failure symptoms in patients with nonobstructive hypertrophic cardiomyopathy"*. [Abstract in section 8.2]

17. Girolami F, Tomberli B, Bardi S, Berti L, Pippucci T, , Cecchi F, Torricelli F, Olivotto I. *"Outcome of hypertrophic cardiomyopathy associated to MYBPC3 mutations: a founder effect in Tuscany".* [Abstract available in section 8.2].

18. Fornaro A, Castelli G, Ciaccheri M, Tomberli B, Olivotto I, Gensini GF. *"Survival of chemotherapy-induced cardiomyopathy in the last two decades"*. [Abstract available in section 8.2]

8.2 Full-text papers and abstracts

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Coronary microvascular dysfunction is an early feature of cardiac involvement in patients with Anderson–Fabry disease

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Aims Male patients with Anderson–Fabry disease (AFD) often exhibit cardiac involvement, characterized by LV hypertrophy (LVH), associated with severe coronary microvascular dysfunction (CMD). Whether CMD is present in patients without LVH, particularly when female, remains unresolved. The aim of the study was to investigate the presence of CMD by positron emission tomography (PET) in AFD patients of both genders, with and without evidence of LVH.

Methods and results We assessed myocardial blood flow following dipyridamole infusion (Dip-MBF) with ^{13}N-labelled ammonia by PET in 30 AFD patients (age 51 $\pm$ 13 years; 18 females) and in 24 healthy controls. LVH was defined as echocardiographic maximal LV wall thickness $\geq$13 mm. LVH was present in 67% of patients ($n = 20$; 10 males and 10 females). Dip-MBF was reduced in all patients compared with controls (1.8 $\pm$ 0.5 and 3.2 $\pm$ 0.5 mL/min/g, respectively, $P < 0.001$). For both genders, flow impairment was most severe in patients with LVH (1.4 $\pm$ 0.5 mL/min/g in males and 1.9 $\pm$ 0.5 mL/min/g in females), but was also evident in those without LVH (1.8 $\pm$ 0.3 mL/min/g in males and 2.1 $\pm$ 0.4 mL/min/g in females; overall $P = 0.064$ vs. patients with LVH). Analysis of variance (ANOVA) for the 17 LV segments showed marked regional heterogeneity of MBF in AFD ($F = 4.46$, $P < 0.01$), with prevalent hypoperfusion of the apical region. Conversely, controls showed homogeneous LV perfusion ($F = 1.25$, $P = 0.23$).

Conclusions Coronary microvascular function is markedly impaired in AFD patients irrespective of LVH and gender. CMD may represent the only sign of cardiac involvement in AFD patients, with potentially important implications for clinical management.

Keywords Fabry disease • Coronary microvascular dysfunction • PET • Early phenotype

Introduction

Anderson–Fabry disease (AFD) is an X-linked disorder of glycosphingolipid catabolism caused by deficient or absent activity of the lysosomal enzyme α-galactosidase A (α-gal A). AFD is considered a rare disease, with a reported prevalence in the general population of ~ 1:117 000.[1,2] Recently, however, newborn screening initiatives have found an unexpectedly high prevalence of AFD, as high as 1 in ~3900 newborns,[3,4] suggesting that previous figures may represent a substantial underestimate.

As a consequence of α-gal A deficiency, progressive accumulation of globotriaosylceramide (Gb3) occurs in various tissues throughout the body, including the endothelium, and is associated with protean clinical manifestations, including renal failure, juvenile stroke, dyspnoea, angina, and sudden cardiac death.[5,6] Cardiac involvement

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has been described in both genders and is mainly characterized by variable degrees of LV hypertrophy (LVH) and interstitial fibrosis.[7] Although commonly classified as a storage disease of the heart, AFD cardiomyopathy is characterized by true LVH, as Gb3 accumulation only accounts for a very limited proportion of cardiac mass increase.[8]

Coronary microvascular dysfunction (CMD) is an important feature of AFD cardiomyopathy, accounting for the considerable prevalence of angina, in the absence of epicardial coronary disease.[8,9] CMD has mostly been described in male AFD patients with LVH.[9–11] However, in patients with sarcomeric hypertrophic cardiomyopathy, a different model of genetically determined LVH, CMD is a diffuse phenomenon within the LV, may involve non-hypertrophied LV walls, and may possibly precede LVH development.[12–15] Whether CMD may be present in AFD patients without LVH remains unresolved. Furthermore, the prevalence and severity of CMD in female AFD patients, generally exhibiting more subtle cardiac manifestation than men, are undefined. Both issues are potentially relevant to early diagnosis of AFD cardiomyopathy and long-term risk stratification.[12–15]

Thus, the present study was undertaken to investigate coronary microvascular function in a cohort of male and female AFD patients at different stages of disease, with and without evidence of LVH, in order to assess whether CMD may occur independently of LVH and in the absence of any other sign of cardiomyopathy. We therefore assessed maximal global and regional myocardial blood flow after dipyridamole (Dip-MBF), as an expression of coronary microvascular function, using positron emission tomography (PET).

Methods

Study population

Thirty patients from 13 families (12 males, 18 females; mean age 51 $\pm$ 13 years) with AFD were included in a cross-sectional study. Diagnosis was based on measurement of α-gal A enzyme activity in leucocytes and confirmed by direct sequencing of the α-gal A gene (*GLA* gene) on genomic DNA isolated from whole blood. Enzyme activity and genetic analysis were assessed as previously described.[16] In two patients (individual II-1 from family 3 and individual III-7 from family 7), the final diagnosis of AFD was achieved by means of endomyocardial biopsy from the right interventricular septum. Between 2006 and 2010, all patients underwent thorough cardiac evaluation by clinical examination, resting ECG, Holter monitoring, and two-dimensional (2D) echocardiography including tissue Doppler analysis.

Coronary microvascular function was assessed by PET as described below. CAD was excluded in AFD patients at the time of enrolment by maximal, symptom-limited treadmill or cycloergometer exercise test, in asymptomatic patients with low pre-test probability (i.e. young and without cardiovascular risk factors), followed by coronary angiography or computed tomography (CT)-angiography in the presence of a positive or dubious test result; patients with a high risk profile and/or angina were directly referred for coronary angiography or CT-angiography. Furthermore, patients with diabetes were excluded. The same study protocol was employed in 24 healthy controls, who were investigated for exclusion of heart disease, and were comparable with the patients in terms of gender and age (13 males and 11 females, $P = 0.55$ vs. patients; age 46 $\pm$ 16 years, $P = 0.23$ vs. patients). Healthy controls had no evidence of cardiomyopathy, with normal ECG, echocardiographic examination, and without common cardiovascular risk factors. The study protocol was approved by the local research ethics committee, and written informed consent in lay Italian language was obtained from each subject included in the study

Echocardiography

Standard echocardiographic studies were performed with commercially available instruments. Clinical diagnosis of LVH was based on the demonstration by 2D echocardiogram of a hypertrophied and non-dilated LV (wall thickness $\geq$ 13 mm) in the absence of another cardiac or systemic disease capable of producing a similar degree of hypertrophy. The LV end-diastolic and end-systolic diameter, left atrial diameter, and magnitude and distribution of LVH were assessed as previously described.[17] In all patients, maximum LV wall thickness values were measured at the time of PET. The LVEF was measured in the standard four-chamber view by the area–length method. Obstruction of the LV outflow was considered present when a peak outflow gradient of $\geq$ 30 mmHg was present under basal conditions. Diastolic function was assessed accordingly to current guidelines.[18]

Positron emission tomography

All cardiac PET scans were performed in the Nuclear Medicine Laboratory (Nuclear Medicine Unit, Department of Clinical Physiopathology, Careggi University Hospital) in Florence between 2006 and 2011, after an appropriate period of pharmacological wash-out for patients receiving pharmacological treatment. Patients were positioned on the couch of the PET scanner (General Electrics Advance PET, Milwaukee, WI, USA) and a 5 min transmission scan was recorded for subsequent attenuation correction of emission data, according to a previously described procedure.[19] Then, near maximal hyperaemia was induced by i.v. administration of dipyridamole (0.56 mg/kg of body weight over 4 min). Three minutes following the end of dipyridamole infusion, a bolus of 370 MBq of nitrogen-13 ammonia (^{13}N-labelled ammonia) diluted in 10 mL of saline solution was injected i.v. over a period of 15–20 s and followed by a 10 mL saline solution flush at a rate of 2 mL/s. A dynamic scan with 15 frames of increasing duration was acquired for 4 min, followed by a prolonged static acquisition of 15 min. Particular care was taken to avoid any patient motion in order to minimize possible misalignment problems between transmission and emission scans. Data were analysed with an operator interactive computer program [PMOD Cardiac Modelling (PCARD), version 3.3, PMOD Technologies, Zurich, Switzerland]. Briefly, anatomic images were reconstructed using the static acquisition, and reoriented according to the heart axis. After reconstruction, the dynamic images were reoriented as well. On the short axis slices, regions of interest were manually drawn including: (i) the right ventricular cavity; (ii) the LV cavity; and (iii) the LV wall from the apex through the base (identified by the appearance of the membranous septum). The regions of interest were edited by the standard PMOD volume of interest (VOI) tool to derive the related VOIs. The three VOIs were then copied on all ^{13}N-labelled ammonia dynamic images to extract the corresponding time–activity curves. The arterial input function was obtained from the LV cavity time–activity curve. The myocardial uptake was derived from the LV wall VOI. Myocardial perfusion was calculated from model fitting of the arterial input function and tissue time–activity curves.[20] The LV wall was divided into 17 segments: septal (apical septal, midinferoseptal, midanteroseptal, basal inferoseptal, and basal anteroseptal), anterior (apical anterior, midanterior, and basal anterior), lateral (apical lateral, midlateral, and basal lateral), inferior (apical inferior, midinferolateral, midinferior, basal inferolateral, and basal inferior), and apical.[21] Mean hyperaemic MBF for the entire left ventricle was obtained by volume-weighted averaging of the 17 LV segment territories (apex, septum, anterior, lateral, and inferior).

All images studies were analysed by one expert observer (R.S.), blinded to patients' genetic, clinical, and echocardiographic data.

Statistical analysis

Data are expressed as mean ± standard deviation. Two-tailed unpaired Student's *t*-test was employed for the comparison of normally distributed data. χ^2 or Fisher's exact test, as appropriate, were utilized to compare non-continuous variables expressed as proportions. Independent determinants of CMD were evaluated by logistic regression analysis. *P*-values are two-sided and considered significant when <0.05. Calculations were performed using the SPSS 12.0 software (Chicago, IL, USA).

Results

Demographic features and mutational status

The mean age of the 30 AFD patients, at the time of PET, was 51 ± 13 years (range 23–75); 12 patients (40%) were male (*Table 1*). Genetic analysis identified 11 distinct mutations in the *GLA* gene (*Table 2*, *Figure 1*); three index patients and seven family members shared the c.644A > G *GLA* gene mutation leading to the p.Asn215Ser amino acid substitution. As expected, mean α-gal A activity in leucocytes was lower in males than in females (2.1 ± 1.4 vs. 21 ± 12 nmol/mg/h, respectively; $P < 0.001$); conversely, mean levels were comparable in patients with and without echocardiographic evidence of LVH (13 ± 13 and 14 ± 14 nmol/mg/h, respectively; $P = 0.75$). The two patients with endomyocardial biopsy, both with echocardiographic evidence of LVH, had typical AFD histological findings.

Overall, 8 had a history of smoking, 5 were current smokers, and 11 had hypercholesterolaemia (*Table 1*). Three patients were obese [body mass index (BMI) >30; 10%], while hypertension, well controlled by medical therapy, was present in 12 patients (40%) without differences between the two subgroups. Evidence of extracardiac involvement was present in 19 patients (63%) (detailed in *Table 2*): 12 patients (40%, 9 males) had proteinuria, 5 males had mild to moderate renal impairment (17%), and 2 patients (6%, 1 male) underwent renal transplantation. Five patients had prior stroke (17%, 2 males), while neuropathic pain was present in 11 patients (37%, 5 males). Nine patients with angina and dubious stress test underwent coronary angiogram or coronary CT-angiogram (as reported in *Table 2*), in order to exclude epicardial CAD. In the remaining patients, coronary disease was excluded by symptom-limited exercise test.

Eleven patients (37%) were receiving enzyme replacement therapy (ERT) at the time of the study, eight patients with LVH and three without LVH. Most patients were on ACE inhibitors/ARBs (57%) and antiplatelet agents (43%), whereas only a minority were on beta-blockers or calcium channel antagonists (17% for each class).

Evidence of Anderson–Fabry disease cardiomyopathy

Twenty of the 30 AFD patients (67%, 10 males, 10 females) had echocardiographic evidence of LVH, with a maximum LV wall thickness value of 20 ± 4 mm (range 13–27 mm). The remaining 10 patients (33%, 2 males, 8 females) had normal LV wall thickness values. Most patients with LVH (76%) had ECG abnormalities compatible with LV hypertrophy/strain. Conversely, only one of the patients without LVH (10%) had an abnormal ECG morphology (*Table 1*).

Compared with patients without LVH, those with LVH were more often males (10/20 or 50% vs. 2/10 or 20% respectively, $P = 0.23$) and more often symptomatic for chest pain or dyspnoea ($P < 0.05$ for both; *Table 1*). Patients with LVH had larger atrial volumes and more frequent evidence of diastolic dysfunction. None of the patients had LV outflow tract obstruction at rest. Of note, patients with LVH were older (56 ± 9 years vs. 42 ± 16 in those without LVH, $P = 0.005$), with a strong direct relationship between age and degree of hypertrophy, particularly in males, suggesting age-related development of AFD cardiomyopathy (*Figure 2A*). Finally, 8 of the 10 patients without LVH (i.e. 7 females and 1 males) showed normal tissue Doppler imaging mitral septal annulus velocities (adjusted per age) and absence of late gadolinium enhancement (LGE) at cardiac magnetic resonance imaging (MRI).

Evidence of coronary microvascular dysfunction by positron emission tomography

Coronary microvascular response to dipyridamole was blunted in all AFD patients, as compared with control subjects (1.8 ± 0.5 and 3.2 ± 0.5 mL/min/g, respectively; $P < 0.001$) (*Figure 3*). Dip-MBF in AFD patients ranged from 0.8 to 2.6 mL/min/g, and was <1.25 mL/min/g, reflecting severe impairment of coronary microvascular function, in 7 patients (23%). Patients with LVH invariably showed marked degrees of CMD (*Figure 2B*). However, patients without LVH also showed blunted Dip-MBF values, ranging from 2.4 to as low as 1.2 mL/min/g. As a result, CMD in patients without LVH was on average milder, but not statistically different, from that of those with LVH (mean 2.0 ± 0.4 vs. 1.6 ± 0.5 mL/min/g, respectively; $P = 0.064$) (*Figure 3*).

Analysis of variance (ANOVA) for the 17 LV segments showed marked regional heterogeneity of MBF in AFD ($P < 0.01$), with prevalent hypoperfusion of the apical region (*Figure 4*). Conversely, controls showed homogeneous perfusion of the LV (patients, $F = 4.46$, $P < 0.01$; controls, $F = 1.25$, $P = 0.23$).

To exclude the possible influence of common CMD risk factors, other than AFD, we compared MBF values between patients with no history of smoking, hypertension, and dyslipidaemia with the rest of the cohort. Patients without risk factors ($n = 12$) showed a similar degree of MBF impairment, as compared with other patients ($n = 18$; MBF = 1.6 ± 0.5 mL/min/g vs. MBF = 1.9 ± 0.4 mL/min/g, respectively; $P = 0.12$). Furthermore, there was no difference in MBF between patients on ERT vs. naïve patients (1.7 ± 0.5 mL/min/g vs. 1.7 ± 05 mL/min/g, respectively; $P = 0.9$).

Impact of gender on microvascular function

Coronary microvascular dysfunction was more severe in males, compared with female AFD patients (1.5 ± 0.4 and 1.9 ± 0.5 mL/min/g, respectively, $P = 0.01$) (*Figure 3*). Unexpectedly, however, a blunted Dip-MBF was also present in the 18 female patients, irrespective of the presence of LVH (1.9 ± 0.5 mL/min/g in females with LVH and 2.1 ± 0.4 mL/min/g in females without LVH; $P = 0.38$). Of note, a 61-year-old female patient without LVH had

Table 1 Baseline and cardiac characteristics of 30 patients with Anderson–Fabry disease

	All patients ($n = 30$)	LVH – ($n = 10$)	LVH + ($n = 20$)	*P*-value
Age, years	51 ± 13	42 ± 16	56 ± 9	0.005
Age <40 years, *n* (%)	6 (10)	5 (50)	1 (5)	0.009
Gender				0.23
Male, *n* (%)	12 (40)	2 (20)	10 (50)	
Female, *n* (%)	18 (60)	8 (80)	10 (50)	
Height, cm	167 ± 8	165 ± 9	168 ± 7	0.42
Weight, kg	69 ± 12	65 ± 10	72 ± 13	0.15
BMI	26 ± 7	27 ± 11	26 ± 4	0.63
Genotype				0.98
p.Asn215Ser (c.644A > G), *n* (%)	10 (33)	3 (30)	7 (35)	
Other mutations, *n* (%)	20 (67)	7 (70)	13 (65)	
Cardiovascular risk factors				
Smoking				
Smokers, *n* (%)	5 (17)	1 (10)	4 (20)	0.64
Ex-smokers, *n* (%)	8 (27)	3 (30)	5 (25)	0.93
Hypercholesterolaemia, *n.* (%)	11 (37)	2 (20)	9 (45)	0.25
Hypertension, *n* (%)	12 (40)	2 (20)	10 (50)	0.23
BMI >30, *n* (%)	3 (10)	1 (10)	2 (10)	0.96
Therapy				
Beta-blockers, *n* (%)	5 (17)	0	5 (25)	0.14
Calcium channel antagonists, *n* (%)	5 (17)	1 (10)	4 (20)	0.64
Amiodarone, *n* (%)	2 (7)	0	2 (10)	0.54
ACE inhibitors, *n* (%)	8 (27)	1 (10)	7 (35)	0.21
ARBs, *n* (%)	9 (30)	1 (10)	8 (40)	0.20
Antiplatelets, *n* (%)	13 (43)	1 (10)	12 (60)	0.017
ERT, *n* (%)	11 (37)	3 (30)	8 (40)	0.70
NYHA class				0.02
I, *n* (%)	17 (57)	9 (90)	8 (40)	
II, *n* (%)	10 (33)	1 (10)	9 (45)	
III/IV, *n* (%)	3 (10)	0	3 (15)	
Dyspnoea, *n* (%)	13 (43)	1 (10)	12 (60)	0.017
Chest pain, *n* (%)				
At rest	2 (7)	1 (10)	1 (5)	0.95
On effort	11 (37)	1 (10)	10 (50)	0.049
Palpitations, *n* (%)	16 (53)	5 (50)	11 (55)	0.99
Syncope, *n* (%)	5 (17)	2 (20)	3 (15)	0.97
NSVT, *n* (%)	3 (10)	0	3 (15)	0.53
PAF, *n* (%)	4 (13)	1 (10)	3 (15)	0.97
Pacemaker, *n* (%)	3 (10)	0	3 (15)	0.53
ECG				
LVH, *n* (%)	14 (52)	1 (10)	13 (76)	0.001
Bradycardia, *n* (%)	11 (37)	4 (40)	7 (35)	0.94
Heart rate, b.p.m.	61 ± 8	61 ± 12	62 ± 7	0.79
Short PR, *n* (%)	3 (10)	1 (10)	2 (10)	0.97
Mean flow <1.25 mL/min/g, *n* (%)	7 (23)	1 (10)	6 (30)	0.37
Echocardiography				
LV max WT, mm	16 ± 6	9 ± 1	20 ± 4	<0.01
IV septum, mm	15 ± 6	9 ± 1	18 ± 5	<0.01
LV posterior wall, mm	12 ± 3	9 ± 1	13 ± 2	<0.01
Left atrium, mm	37 ± 7	31 ± 5	40 ± 6	<0.01
Left atrium volume, mm/m^2	39 ± 13	29 ± 9	45 ± 11	<0.01

Continued

Table 1 *Continued*

	All patients ($n = 30$)	LVH − ($n = 10$)	LVH + ($n = 20$)	*P*-value
LV telediastolic diameter, mm	48 ± 6	48 ± 5	49 ± 6	0.75
LV telesystolic diameter, mm	27 ± 8	27 ± 5	27 ± 7	0.86
LV telediastolic volume, mL	99 ± 32	91 ± 19	102 ± 37	0.40
LV telesystolic volume, mL	37 ± 20	33 ± 13	39 ± 23	0.42
EF, %	63 ± 10	65 ± 9	63 ± 10	0.62
Diastolic pattern				0.03
Normal, *n* (%)	6 (20)	6 (60)	0	
Delayed relaxation, *n* (%)	12 (40)	4 (40)	8 (40)	
Pseudonormal, *n* (%)	11 (37)	0	11 (50)	
Restrictive, *n* (%)	1 (3)	0	1 (5)	
LVOT obstruction, *n* (%)	0	0	0	N/A
Mitral regurgitation (mild or moderate)	16 (53)	4 (40)	12 (60)	0.44
Aortic regurgitation (mild or moderate)	5 (17)	2 (20)	3 (15)	0.92

Data are shown as mean ± SD or *n* (%).
BMI, body mass index; ERT, enzyme replacement therapy; IV, interventricular; LVH −, patients without LV hypertrophy; LVH +, patients with LV hypertrophy; LV max WT, LV maximal wall thickness; LVOT, LV outflow tract obstruction; N/A, not applicable; NSVT, non-sustained ventricular tachycardia; PAF, paroxysmal atrial fibrillation.

extreme degrees of coronary flow impairment (Dip-MBF = 1.2 mL/min/g) (family 6, subject II-1 in *Table 2*). An inverse relationship between mean Dip-MBF and degree of LVH, suggesting more severe CMD in the presence of increasing LVH, was present only in males (*Figure 2B*). The 10 patients with the p.Ans215Ser *GLA* gene mutation showed significant blunting of MBF as compared with patients with other mutations (1.5 ± 0.5 vs. 1.9 ± 0.5 mL/min/g, respectively; $P = 0.019$), despite a similar degree of hypertrophy (maximum LV wall thickness 18 ± 8 mm vs. 15 ± 5 mm, respectively, $P = 0.28$).

Discussion

Coronary microvascular dysfunction is independent of left ventricular hypertrophy in Anderson–Fabry disease

The present study demonstrates that microvascular function is markedly impaired in AFD patients, irrespective of any other evidence of cardiac involvement. In our cohort, all AFD patients with normal echocardiographic findings exhibited blunting of the vasodilator response to dipyridamole, reflecting impaired microvascular function, with a mean Dip-MBF of only 2.0 ± 0.4 mL/min/g, compared with 3.2 ± 0.5 mL/min/g in normal controls, i.e. a 60% reduction in coronary flow. Such a degree of CMD was only slightly milder than that observed in patients with clear evidence of AFD cardiomyopathy and LVH. Indeed the most severe impairment in microvascular function was observed in a 61-year-old female patient, without evidence of LVH (Dip-MBF 1.2 mL/min/g).

Of note, while MBF was globally impaired in AFD hearts, there was marked heterogeneity in flow, with prevalent hypoperfusion of the apical region of the left ventricle, suggesting a regional nature of cardiac involvement. While counterintuitive in a systemic storage disease, the novel idea of a non-homogeneous cardiac involvement in AFD is in line with prior observations in a number of cardiomyopathies, and particularly those related to storage or infiltrative disorders, such as, for example, amyloidosis.[22] The mechanisms accounting for such regionally heterogeneous manifestations remain unresolved.

Altogether, these findings point to CMD as a constant manifestation of AFD, which may be present independent of other instrumental evidence of cardiac involvement. In males, the correlation between LV wall thickness and maximal MBF, and the direct relationship of LV wall thickness to age, both suggest that a reduced coronary maximal blood flow may precede development of LVH (*Figure 2*). This concept is novel and strongly supported by the young age of patients with CMD in the absence of LVH. Furthermore, AFD patients without LVH and CMD generally had none of the other features that are considered very early disease manifestations, such as reduced diastolic mitral annulus velocities or presence of LGE.[23,24] Nevertheless, longitudinal studies are required to understand whether flow abnormalities can indeed be considered to represent initial cardiac involvement followed by overt cardiomyopathy in AFD patients.

The consistent and severe blunting of MBF in patients with the p.Ans215Ser *GLA* gene mutation may suggest a role for genetic status in determining the severity of MBF. Furthermore, the concept of the possible influence of genetic status on microvascular function has been proved in another more common genetic cardiomyopathy (i.e. hypertrophic cardiomyopathy).[25] However, in the present study, the relatively small number of patients did not allow a meaningful comparison between individual mutations.

Coronary microvascular dysfunction in female Anderson–Fabry disease patients

In our cohort, females with LVH showed blunted Dip-MBF, although to a less severe degree than males (1.9 ± 0.5 and 1.5 ± 0.4 mL/min/g,

Table 2 Clinical and genetic features of the study population at the time of positron emission tomography

Family	Genotype (effect on protein)	Subject	Sex	Age			α-Gal	ERT	Symptoms (NYHA class)	LVH	Max LVWT	EF	Dip-MBF	CAD exclusion	Major organ involvement
				At clinical onset	At diagnosis	At PET									
1	p.Met1Val (c.1 A > G)	III-1	M	4	28	29	0.9	Yes	Palpitations (1)	Yes	16	67	2.4	Stress test	Proteinuria, acroparaesthesia, abdominal pain
		II-1	F	16	47	48	46	Yes	Dyspneoa, palpitations, syncope, angina (2)	Yes	16	62	2.3	Coronary angiogram	Acroparaesthesia
2	p.Trp44X (c.131 G > A)	III-1	M	12	52	54	0.01	Yes	Dyspnoea, palpitations (2)	Yes	23	70	1.1	Coronary CT-angiogram	Acroparaesthesia, RI, proteinuria
		III-2	F	-	39	41	11.8	No	Palpitations (1)	No	9	60	1.8	Stress test	None
3	p.Arg112Cys (c.334 C > T)	II-1	M	11	34	52	1.9	Yes	Angina (1)	Yes	23	66	0.8	Coronary angiogram	Acroparaesthesia stroke, renal transplant
4	p.Ala143Thr (c.427 G > A)	III-2	F	-	40	42	5.8	No	Palpitations (1)	No	8	64	2.4	Stress test	None
5	p.Asn215Ser (c.644 A > G)	II-1	M	44	56	60	1.82	No	None (1)	Yes	25	59	1.7	Coronary CT-angiogram	None
6	p.Asn215Ser (c.644 A > G)	II-1	F	-	56	60	13	No	Dyspnoea, palpitations (2)	No	11	67	1.2	Stress test	None
		II-2	M	48	55	55	3.4	No	Dyspnoea, angina (2)	Yes	28	58	1.2	Coronary angiogram	RI, proteinuria
		II-3	F	N/A	53	56	16	No	Dyspnoea (2)	Yes	13	70	0.9	Stress test	Acroparaesthesia
		II-4	M	N/A	46	53	4.2	No	Dyspnoea, palpitations (2)	Yes	27	62	1.0	Stress test	RI, proteinuria
		II-5	M	–	51	48	3.6	No	Dyspnoea, angina (2)	Yes	22	70	1.3	Stress test	None
		III-3	F	-	24	27	44	No	None (1)	No	7	76	2.2	Stress test	None
		III-5	F	20	25	31	5.3	No	Syncope, palpitations (1)	No	9	67	2.3	Stress test	Acroparaesthesia
7	p.Asn215Ser (c.644 A > G)	III-7	M	59	69	69	3.2	No	Palpitations, dyspnea, angina (3)	Yes	23	33	1.2	Coronary angiogram	Proteinuria
		III-12	M	50	68	69	2.8	No	Dyspnea, angina (2)	Yes	23	71	1.5	Stress test	Proteinuria, RI, stroke
8	p.Arg220X (c.658 C > T)	II-2	F	22	37	47	26.6	Yes	None (1)	Yes	21	82	1.6	Coronary angiogram	Renal transplant
9	p.Leu243Ser (c.728 T > C)	III-1	F	N/A	63	62	26	No	Palpitations, dyspnoea (2)	Yes	16	62	2.3	Stress test	None
		III-2	F	36	56	57	22	No	Dyspnoea, palpitations, angina (3)	Yes	23	47	2.2	Stress test	Proteinuria
10	p Asp299Gly (c.896 A > C)	II-1	F	N/A	66	68	21	No	None (1)	Yes	17	58	1.9	Stress test	None
		III-1	F	-	49	49	31	No	Palpitations (1)	No	11	59	1.9	Stress test	None
		III-2	M	18	46	49	0.01	Yes	None (1)	Yes	15	61	2.0	Stress test	Proteinuria, RI, acroparaesthesia, abdominal pain
11	p.Arg301Pro (c.902 G > C)	III-2	F	16	49	63	13.5	Yes	Palpitations, dyspnoea, syncope, angina (2)	Yes	21	58	1.6	Stress test	Proteinuria, acroparaesthesia, strokes
		III-4	F	N/A	46	56	31.6	No	None (1)	Yes	14	63	2.6	Stress test	None
		III-5	F	N/A	55	62	18.2	No	Dyspnoea (2)	Yes	15	63	1.4	Stress test	Stroke
		IV-1	M	25	30	40	0.9	Yes	None (1)	No	10	59	2.0	Stress test	Proteinuria
12	p.Arg301X (c.901 C > T)	I-1	F	–	70	75		No	Palpitations (1)	No	9	77	2.2	Coronary angiogram	None

Continued

13	c.1235_1236 delCT	II-1	F	15	40	54	6.6	Yes	Dyspnoea, palpitations (2)	Yes	13	76	1.8	Coronary CT-angiogram	Acroparaesthesia, strokes, abdominal pain
		III-1	M	4	15	28	2.4	Yes	None (1)	No	10	53	1.5	Stress test	Acroparaesthesia, abdominal pain, proteinuria
		III-3	F	14	10	23	13.8	Yes	None (1)	No	8	63	2.3	Stress test	Acroparaesthesia, abdominal pain

α-Gal = α-galactosidase A activity assayed on leucocytes (normal values 20–60 nmol/mg/h); CT-angiogram, computed tomography angiogram; Dip-MBF, mean myocardial blood flow following dipyridamole infusion; ERT, enzyme replacement therapy; LVH, left ventricular hypertrophy; Major organ involvement, only kidney (RI, renal impairment diagnosed as glomerular filtration rate < 60 mL/min), peripheral and central nervous system involvement are reported in this table; Max LVWT, maximal left ventricular wall thickness; N/A, age at onset unknown (diagnosis made by family screening).

respectively; $P = 0.01$). In addition, and rather unexpectedly, women without LVH also had considerable degrees of CMD, representing the only evidence of AFD in some of these individuals. As a result, there should be heightened awareness of the consideration of cardiac involvement in female patients with AFD, even in the absence of an echocardiographic phenotype. Although AFD is an X-linked disease, female patients are well known to develop the clinical manifestations of the disease, which can be severe and determine outcome.[26] Cardiomyopathy is not an exception to this rule, and has been described in women; however, cardiac manifestations generally have a later onset and are less marked than those occurring in men (*Figure 4*).[27,28]

Potential mechanisms of coronary microvascular dysfunction and implication for management

There are several potential pathophysiological mechanisms underlying coronary blood flow impairment in AFD, and these include those mediated by LVH (reduced capillary density, extravascular compression forces) as well as those directly affecting the microvasculature (endothelial dysfunction due to Gb3 storage, nitric oxide pathway dysregulation, or microvascular remodelling).[13] However, in patients without LVH, only the latter are present, suggesting that LVH *per se* is indeed a likely contributor to CMD, rather than a cause.

Despite considerable advances in our understanding of AFD, the mechanisms leading to LVH are not well understood. Storage of Gb3 within cardiac myocytes accounts for a small amount of the whole LV mass, which is mainly represented by true myocardial hypertrophy.[8] One hypothesis is that intracellular accumulation of Gb3 may disturb cardiac energetic metabolism, representing an early trigger for the activation of the intracellular signalling pathways leading to hypertrophy, fibrosis, apoptosis, and necrosis.[29] Furthermore, microvascular dysfunction and chronic hypoperfusion, due to Gb3 accumulation in endothelial cells, may also play a crucial role in the activation and perpetuation of these pathophysiological processes, even at early stages.[13]

The issue of identification of early organ damage has become critical following the introduction in clinical practice of ERT and novel therapeutic molecules, including pharmacological chaperone therapy.[30–33] To date, evidence of benefit of ERT in AFD patients with overt signs of cardiomyopathy and LVH is disappointing, and earlier timing of treatment initiation has been advocated in order to improve efficacy.[11,30]

Thus, early detection of subclinical cardiac involvement, as allowed by PET studies of microvascular function, may become a critical element in clinical decision-making especially in young AFD patients. In particular, CMD may represent a viable treatment target, potentially relevant to prevention of disease progression and outcome.[30] In the future, the advent of cardiac magnetic resonance (CMR), by virtue of its wider availability and more favourable safety profile compared with PET, may allow large-scale investigation of CMD in AFD patients. To date, however, quantitative assessment of microvascular flow by CMR is time-consuming and largely limited to research purposes.[15]

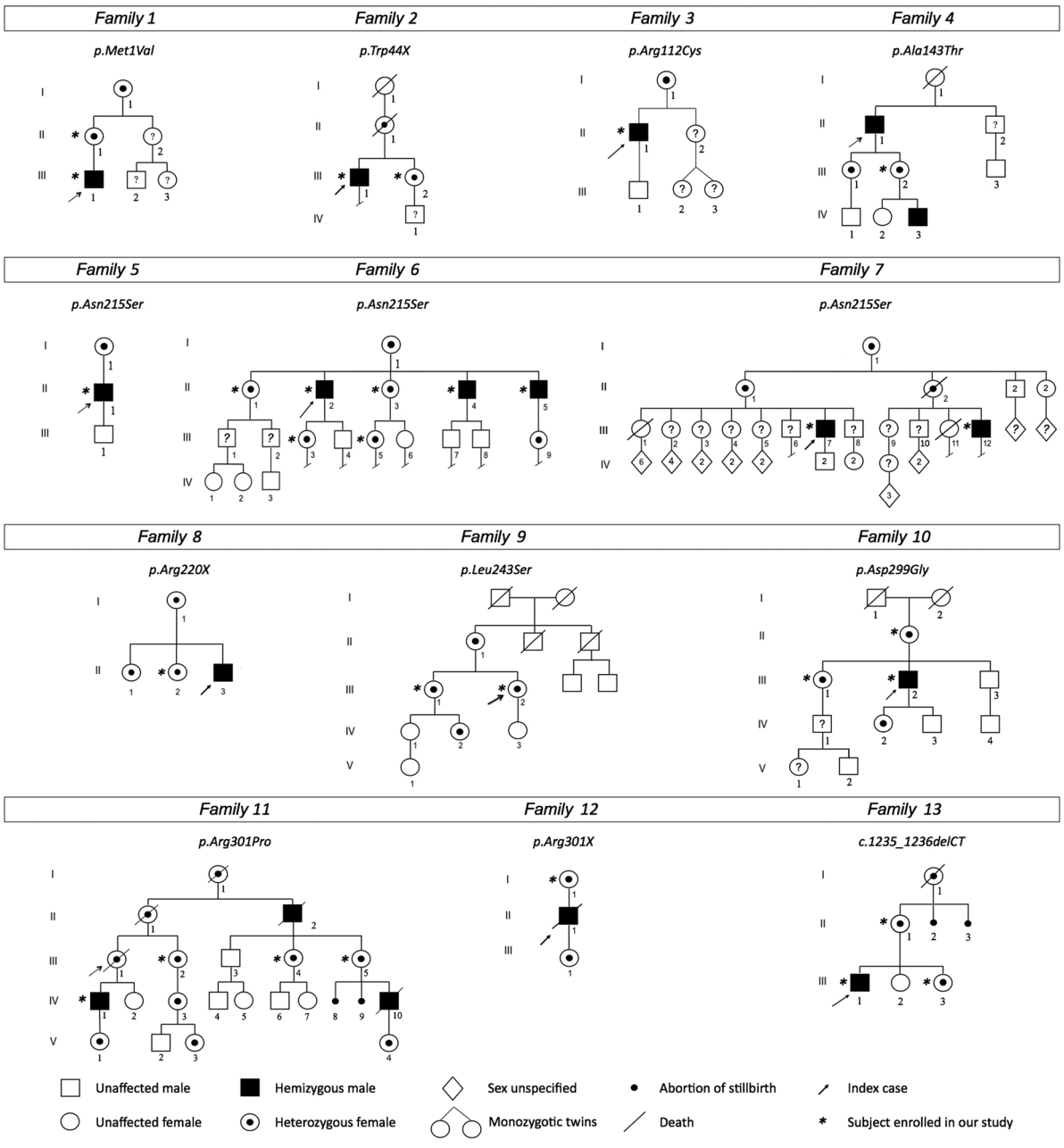

Figure 1 Pedigree of the 13 index patients.

Furthermore, is it possible to hypothesize that the severity of CMD may be an important predictor of adverse outcome, as for other genetically determined cardiomyopathies.[12,34]

Limitations of the study

An unavoidable limitation of the present study lies in the small sample size and heterogeneity of our study population. Selecting a more homogeneous study cohort, by excluding patients with co-morbidities or cardiovascular risk factors, would not have been feasible in a rare and multisystemic disorder such as AFD. Thus, myocardial blood flow may have been affected in our cohort by the interplay of co-morbidities such as hypertension, smoking, or dyslipidaemia, as well as ERT itself. This may have potentially led to overestimation of CMD in our patients with regard to the healthy controls, in whom none of the conventional cardiovascular risk factors was present. The presence of epicardial coronary disease was not systematically excluded by coronary angiogram or coronary CT-angiogram in all patients, based on radioprotection and safety considerations. Nevertheless, none of our enrolled AFD patients, at >3 years average from the execution of PET studies, has been found to have significant CAD during follow-up.

While we acknowledge this unavoidable bias, however, MBF values in AFD with no history of smoking, hypertension, and dyslipidaemia,

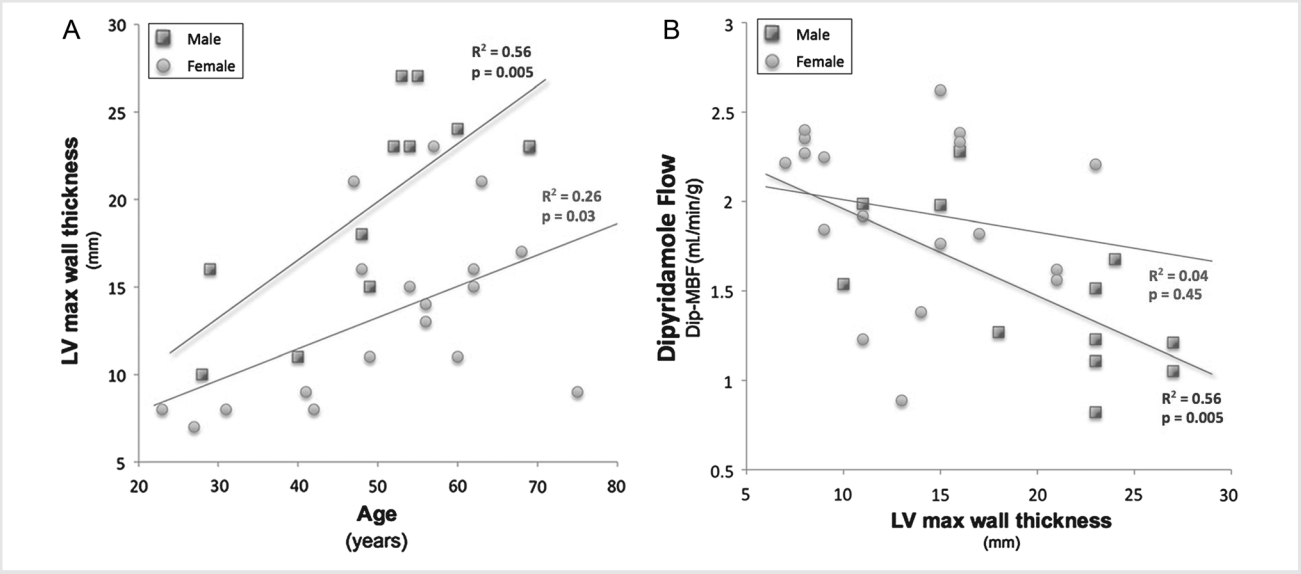

Figure 2 Relationship between LV wall thickness and myocardial flow. Linear regression analysis showed a direct relationship between age and degree of LV hypertrophy (LVH) (*A*) and between degree of LVH and myocardial blood flow following dipyridamole infusion. (*B*). Both relationships were stronger in males, suggesting that the development of Anderson–Fabry cardiomyopathy is age related and that coronary microvasculature dysfunction may precede LVH.

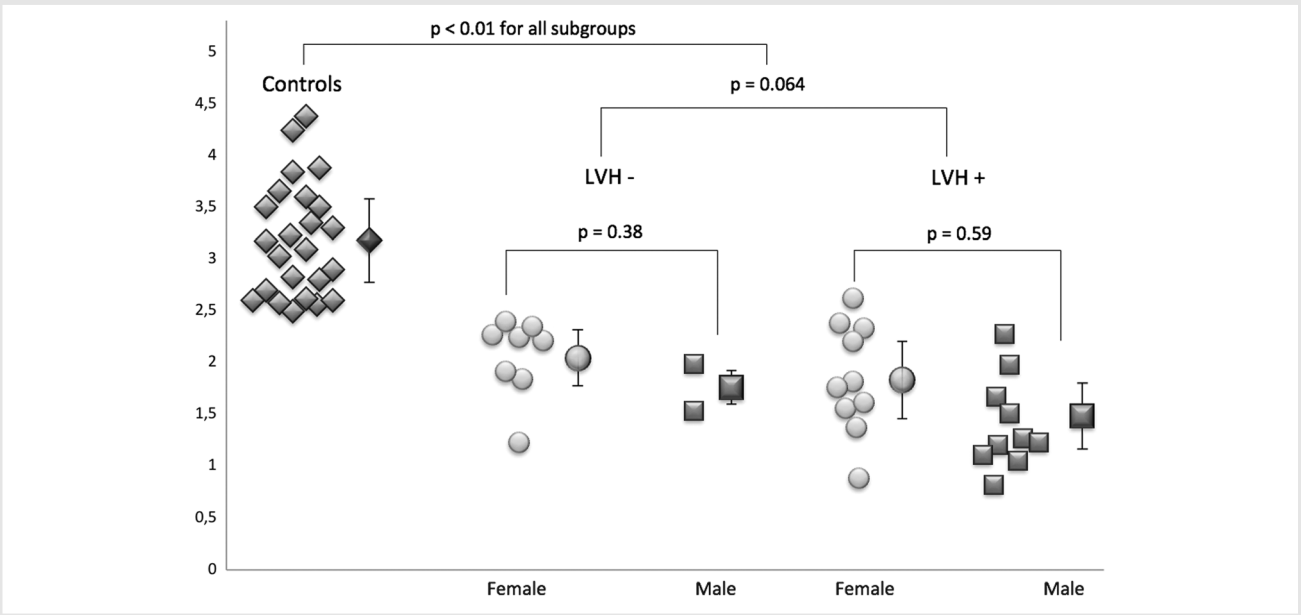

Figure 3 Comparison of microvascular dysfunction in patients with Anderson–Fabry disease (AFD), based on the presence of LV hypertrophy (LVH) and gender. Myocardial blood flow following dipyridamole infusion is markedly impaired in AFD patients compared with control subjects. Values are shown as mean ± SD.

ideally representing 'pure' AFD patients, were comparable with the rest of the cohort. Likewise, MBF in patients who were on ERT at the time of the study was comparable with that of those who were untreated at that time, consistent with previous studies which confirmed the questionable efficacy of ERT in patients with clear-cut cardiomyopathy.[9,11,30] Thus, our data are consistent with a disproportionate microvascular involvement in AFD, overruling the effect of these potential environmental confounders.

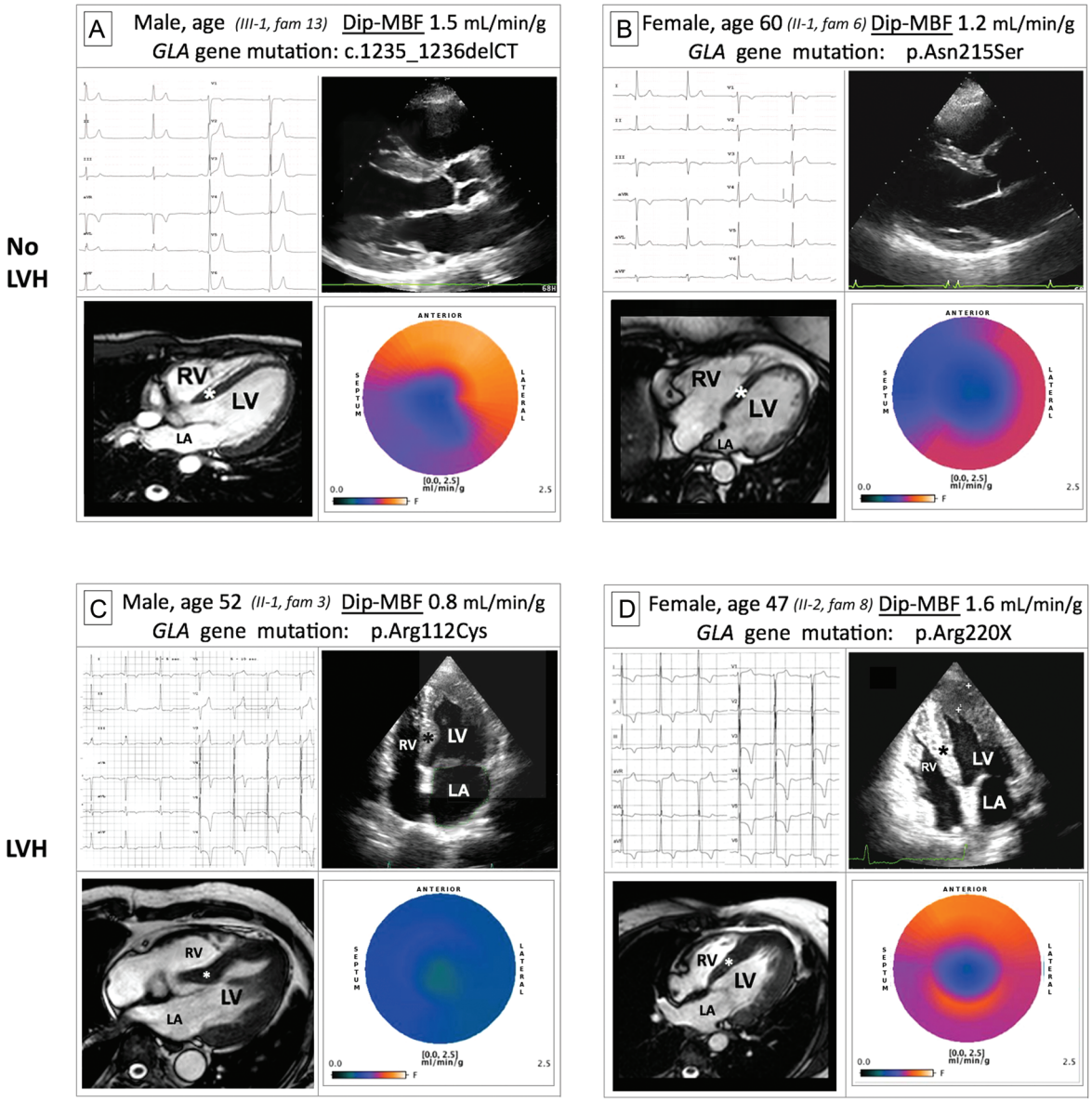

Figure 4 Relationship of coronary microvasculature dysfunction (CMD) to phenotype in individual patients with Anderson–Fabry disease (AFD). (*A*) A 28-year-old male with extreme sinus bradycardia (36 b.p.m.) and normal interventricular septal thickness values (*echocardiographic parasternal long axis and cardiac magnetic resonance four-chamber views). Colour-coded polar map obtained by positron emission tomography (PET) after dipyridamole infusion showing regional CMD, with relatively preserved flow in the antero-lateral region (family 13, subject III-1; *Table 2*). (*B*) A 60-year-old female with normal ECG, without normal LV wall thickness values. The polar map shows severe CMD, despite normal LV wall thickness values (family 6, subject II-1; *Table 2*). (*C*) A 52-year-old male with marked ECG abnormalities, severe LV hypertrophy (LVH) (*echocardiographic apical four-chamber view and cardiac magnetic resonance four-chamber view). The polar map shows severe CMD (family 3, subject II-1; *Table 2*). (*D*) A 47-year-old female with markedly abnormal ECG, and severe LVH. The polar map shows relatively preserved microvascular function, despite the LVH (family 8, subject II-2; *Table 2*). Dip-MBF, myocardial blood flow following dipyridamole infusion,

Comparably, due to the small and heterogeneous patient cohort and the cross-sectional design of the study, it is not possible to extrapolate whether CMD represents an early phase of cardiac involvement in AFD, invariably followed by onset of a clear-cut cardiomyopathy over time. Likewise, it is not possible at this stage to envisage whether these abnormalities are susceptible to regression with specific therapy; both such hypotheses need to be specifically addressed in longitudinal studies. However, our findings imply that a normal echocardiogram cannot exclude deterioration of microvascular function related to the disease, raising important issues regarding risk stratification and management.

Conclusions

Coronary microvascular function is markedly impaired in AFD patients irrespective of LVH and gender. CMD may represent the first sign of cardiac involvement in patients who would be otherwise considered unaffected. These findings suggest that coronary blood flow impairment may precede the development of cardiac hypertrophy in AFD, representing an important element for patient clinical management and decision-making in this challenging disease.

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Obesity and its Association to Phenotype and Clinical Course in Hypertrophic Cardiomyopathy

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Objectives This study sought to assess the impact of body mass index (BMI) on cardiac phenotypic and clinical course in a multicenter hypertrophic cardiomyopathy (HCM) cohort.

Background It is unresolved whether clinical variables promoting left ventricular (LV) hypertrophy in the general population, such as obesity, may influence cardiac phenotypic and clinical course in patients with HCM.

Methods In 275 adult HCM patients (age 48 ± 14 years; 70% male), we assessed the relation of BMI to LV mass, determined by cardiovascular magnetic resonance (CMR) and heart failure progression.

Results At multivariate analysis, BMI proved independently associated with the magnitude of hypertrophy: pre-obese and obese HCM patients (BMI 25 to 30 kg/m^2 and >30 kg/m^2, respectively) showed a 65% and 310% increased likelihood of an LV mass in the highest quartile (>120 g/m^2), compared with normal weight patients (BMI <25 kg/m^2; hazard ratio [HR]: 1.65; 95% confidence interval [CI]: 0.73 to 3.74, p = 0.22 and 3.1; 95% CI: 1.42 to 6.86, p = 0.004, respectively). Other features associated with LV mass >120 g/m^2 were LV outflow obstruction (HR: 4.9; 95% CI: 2.4 to 9.8; p < 0.001), systemic hypertension (HR: 2.2; 95% CI: 1.1 to 4.5; p = 0.026), and male sex (HR: 2.1; 95% CI: 0.9 to 4.7; p = 0.083). During a median follow-up of 3.7 years (interquartile range: 2.5 to 5.3), obese patients showed an HR of 3.6 (95% CI: 1.2 to 10.7, p = 0.02) for developing New York Heart Association (NYHA) functional class III to IV symptoms compared to nonobese patients, independent of outflow obstruction. Noticeably, the proportion of patients in NYHA functional class III at the end of follow-up was 13% among obese patients, compared with 6% among those of normal weight (p = 0.03).

Conclusions In HCM patients, extrinsic factors such as obesity are independently associated with increase in LV mass and may dictate progression of heart failure symptoms. (J Am Coll Cardiol 2013;62:449–57) © 2013 by the American College of Cardiology Foundation

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease, characterized by heterogeneous phenotypic expression with extreme diversity in the pattern and extent of left ventricular hypertrophy (LVH), due to molecular pathways and triggers that remain largely unexplained (1–5). In the vast majority of genotype-positive patients, HCM is associated with mutations in genes encoding proteins of the cardiac sarcomere, most commonly beta-myosin heavy chain and myosin-binding protein C (1–3). While these molecular defects are considered responsible for the development of LVH, there is currently no conclusive evidence to explain the variability in phenotypic expression of HCM, ranging from massive degrees to absence of LVH even within the same family (1,4–6).

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Among several hypotheses, the interplay of modifier genes and environmental factors has been commonly offered as a potential explanation for phenotypic diversity (7,8). To date, however, the possibility of an environmental modulation of the HCM phenotype remains speculative, and even the impact

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Abbreviations and Acronyms

BMI = body mass index
CMR = cardiovascular magnetic resonance
HCM = hypertrophic cardiomyopathy
HR = hazard ratio
LGE = late gadolinium enhancement
LV = left ventricular
LVH = left ventricular hypertrophy
NYHA = New York Heart Association

of an obvious candidate variable such as obesity, known to promote LVH in the general population, is unresolved (9–15). In addition, it is unknown whether the adverse metabolic and hemodynamic effects of obesity, to which HCM patients may be exposed during the long-term course of their disease, ultimately affect symptomatic status and prognosis (11,12,16–18). Therefore, the present study was designed, in a consecutive multicenter cohort studied with cardiac magnetic resonance (CMR), to assess the impact of body mass index (BMI) on the phenotype, as well as clinical course, of HCM.

Methods

Study population. The study cohort comprised 275 adult patients with HCM (age >18 years, mean 48 ± 14 years at study entry; 70% male, maximum left ventricular (LV) wall thickness 21 ± 5 mm) consecutively referred for CMR studies between January 2005 and June 2008 at 3 participating referral centers in the United States and Italy: Minneapolis Heart Institute Foundation (Minneapolis, Minnesota; n = 168); Tufts Medical Center (Boston, Massachusetts; n = 45); and Careggi University Hospital (Florence, Italy; n = 62). Diagnosis of HCM was based on 2-dimensional echocardiographic evidence of a hypertrophied, nondilated LV (maximal wall thickness ≥15 mm), in the absence of another cardiac or systemic disease that could produce the magnitude of hypertrophy evident (1,3). We excluded significant atherosclerotic coronary artery disease (>50% stenosis in a major artery) by virtue of 2 specific clinical or CMR criteria: 1) no study patient experienced an acute coronary event associated with increased cardiac enzymes or Q waves on electrocardiogram; and 2) in all patients with late gadolinium enhancement (LGE) distributed in a single coronary vascular territory, hemodynamically significant coronary artery disease was excluded by arteriography or computed tomography angiogram. Furthermore, patients with prior cardiac surgery (including septal myectomy), alcohol septal ablation, and chronic renal failure were excluded (3). The study protocol was approved by the respective Internal Review Boards or research ethics committees of each institution, and written inform consent was obtained from each subject.

Definitions. Body mass index was calculated as weight/(height · height) and expressed in kg/m^2. Patients were classified as normal weight (BMI range <25 kg/m^2), pre-obese (25 to 30 kg/m^2), and obese (>30 kg/m^2), according to existing guidelines (14). Type 2 diabetes was defined (and treated) according to standard guidelines (12,18).

Systemic hypertension was diagnosed based on resting blood pressure values >140/90 mm Hg on ≥3 different examinations and treated medically to optimize blood pressure control, as per standard international guidelines (18). All patients with hypertension had a diagnosis of HCM based on 1 or more of the following criteria: 1) HCM-causing sarcomere gene mutation or family history of HCM; 2) onset of hypertension occurring years after the diagnosis of HCM; 3) maximum LV wall thickness exceeding that expected by hypertension alone (i.e., >20 mm); 4) presence of marked mitral leaflet elongation (19); 5) dynamic LV outflow obstruction (≥30 mm Hg) under resting conditions (20); and 6) distribution of LGE by contrast CMR consistent with HCM (i.e., preferentially mid-wall or transmural, and not confined to a single coronary vascular territory) (3,5,21).

Echocardiography. Echocardiographic studies were performed with commercially available instruments. Left ventricular hypertrophy was assessed with 2-dimensional echocardiography, and the site and extent of maximal wall thickness were identified. Maximal end-diastolic LV wall thickness was taken as the dimension of greatest magnitude at any site within the chamber. Left ventricular outflow obstruction, due to mitral valve systolic anterior motion and mitral-septal contact, was identified by a peak instantaneous outflow gradient ≥30 mm Hg occurring under basal conditions (n = 57) (20). Two hundred and eighteen patients were nonobstructive at rest (basal gradient <30 mm Hg), of whom 105 (age 43 ± 13 years, 72% males) underwent maximal symptom-limited exercise echocardiography, as previously described (18); 50 developed dynamic gradients ≥30 mm Hg during effort or recovery (range 48 to 155 mm Hg), and were considered to have provokable outflow obstruction (20).

CMR. All CMR examinations were performed using commercially available scanners (Philips ACS-NT 1.5-T Gyroscan-Intera, Best, the Netherlands) and a commercial cardiac coil. Electrocardiographic gated, steady-state, free precession breath-hold cines in sequential 10-mm short-axis slices (no gap) were acquired starting parallel to the atrioventricular ring and covering the entire ventricle. Left ventricular end-diastolic and end-systolic volumes, left ventricular mass and wall thickness were calculated with commercially available work stations (View Forum, Philips Medical System, Best, the Netherlands) (19,21).

For calculation of LV mass, the endocardial and epicardial borders of the left ventricle were manually planimetered on successive short-axis cine images at end-diastole. The most basal slice at end-diastole was visually inspected and, if ventricular myocardium was present, it was planimetered and included in the mass calculation. If myocardium but no intracavitary blood pool was present on the most apical slice, it was included in the mass calculation by planimetering only the epicardial border. Particular care was taken to avoid including papillary muscles in the LV mass calculation. Left ventricular mass was derived by the summation of

discs method and multiplying myocardial muscle volume by 1.05 g/cm^3 (21). Left ventricular mass was indexed to body surface area. Maximum end-diastolic LV wall thickness was taken as the dimension of greatest magnitude at any site within the LV wall. CMR measurements were performed by an experienced investigator at each center, blinded to the results of echocardiography. The presence of LGE was assessed by visual inspection 15 min after intravenous administration of 0.2 mmol/kg gadolinium-diethylenetriaminepentaacetic acid (Magnevist, Schering, Berlin, Germany) with breath-held segmented inversion-recovery sequence (inversion time 240 to 300 ms), which was acquired in the same views as the cine images (21).

Statistical methods. Continuous variables were expressed as mean ± SD, or median or interquartile range, as appropriate. For the comparison of normally distributed variables, we employed Student *t* test and 1-way analysis of variance followed by Bonferroni post-hoc test, as appropriate. Chi-square test (not adjusted for multiple comparisons) was utilized to compare noncontinuous variables expressed as proportions; Fisher exact test was employed when 1 or more cells in the comparison table had an expected frequency of <5. When comparing noncontinuous variables among the 3 BMI classes, an overall p value was obtained using the Monte Carlo method; when the p value was <0.05, individual comparisons between the subgroups were then assessed by cross-tabulation analysis.

Clinical features independently associated with increased LV mass index were assessed by multivariate logistic regression analysis using the stepwise (forward conditional) method. Therefore, the multivariate model was ultimately constructed only with the variables that proved significant at univariate analysis. Survival was assessed by Cox proportional hazards regression. The survival curve was constructed according to the Kaplan-Meier method, and comparisons were performed using the log-rank test; p values are 2-sided and considered significant when <0.05. Calculations were performed with SPSS 12.0 software (Chicago, Illinois).

Results

Prevalence of obesity. The 275 HCM patients had an average BMI of 29.1 ± 6.1 kg/m^2, ranging from 16.2 to 49.3 kg/m^2. Sixty-nine patients (25%) were in the normal

Table 1 Clinical and Echocardiographic Features of the 275 HCM Patients in Relation to BMI

	Overall	Normal BMI <25 kg/m^2	Pre-Obese BMI 25–30 kg/m^2	Obese BMI >30 kg/m^2	p Value
n	275	69 (25%)	105 (38%)	101 (37%)	
Male	192 (70%)	32 (46%)	81 (77%)	79 (78%)	<0.001
Age at diagnosis, yrs	43 ± 14	41 ± 15	45 ± 13	44 ± 13	0.257
Age at CMR, yrs	48 ± 14	46 ± 14	50 ± 14	49 ± 13	0.123
Body surface area, m^2	1.97 ± 0.25	1.73 ± 0.18	1.99 ± 0.2	2.11 ± 0.23†	<0.001
BMI, kg/m^2	29.1 ± 6.1	22.3 ± 2.1	27.4 ± 1.4	35.4 ± 4.8†	<0.001
Height, m	1.71 ± 0.12	1.68 ± 0.09	1.75 ± 0.12†	1.68 ± 0.14	<0.001
Weight, kg	85 ± 19	63 ± 9	84 ± 11	101 ± 16†	<0.001
NYHA functional class at first evaluation					
I	149 (54%)	37 (54%)	64 (61%)	48 (47%)	overall
II	67 (24%)	19 (27%)	22 (21%)	26 (26%)	0.183
III	15 (5%)	3 (4%)	3 (3%)	9 (9%)	
Syncope	57 (20%)	10 (14%)	26 (25%)	21 (21%)	0.263
Atrial fibrillation	30 (11%)	4 (6%)	12 (11%)	14 (14%)	0.248
Hypertension	75 (27%)	8 (12%)	29 (28%)	38 (38%)	0.001
Type II diabetes	14 (5%)		4 (4%)	10 (10%)	0.012
Hypercholesterolemia	84 (31%)	9 (13%)	40 (38%)	35 (35%)	0.001
Echocardiography					
Left atrial diameter, mm	44 ± 8	42 ± 7	44 ± 8	46 ± 7*	0.032
LV end-diastolic diameter, mm	45 ± 6	43 ± 6	45 ± 6	46 ± 6*	0.025
Maximum LV wall thickness, mm	21 ± 5	22 ± 6	21 ± 5	21 ± 5	0.272
With LV outflow obstruction	107 (39%)	15 (22%)	41 (39%)	51 (50%)*	0.001
Medical treatment					
Beta-blockers	151 (55%)	28 (40%)	62 (59%)	61 (60%)	0.427
Verapamil	52 (19%)	9 (13%)	22 (21%)	21 (21%)	0.578
Amiodarone	15 (5%)	4 (6%)	6 (6%)	5 (5%)	0.850
Disopyramide	10 (4%)	2 (3%)	4 (4%)	4 (4%)	0.613
Diuretics	29 (10%)	2 (3%)	9 (8%)	18 (18%)*	<0.001
ACE inhibitors/sartans	40 (14%)	4 (6%)	14 (13%)	22 (21%)*	<0.01
Warfarin	14 (5%)	0	5 (5%)	9 (9%)	0.155

Values are n (%) or mean ± SD. *p < 0.05 versus the other 2 groups; †p < 0.05 versus normal.

ACE = angiotensin-converting enzyme; BMI = body mass index; CMR = cardiac magnetic resonance; HCM = hypertrophic cardiomyopathy; LV = left ventricular; NYHA = New York Heart Association.

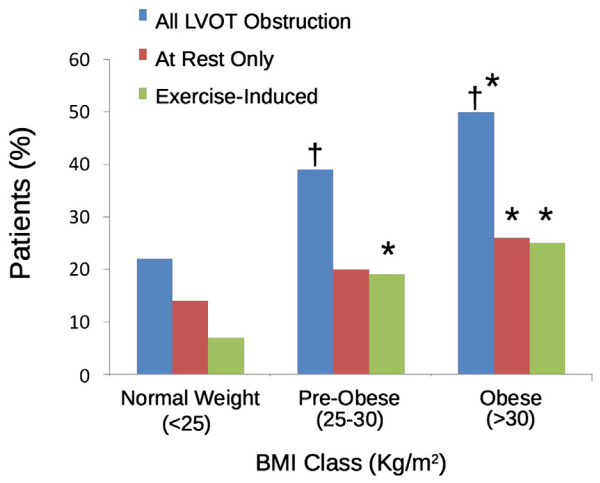

Figure 1 **Prevalence of LV Outflow Obstruction in Relation to BMI**

Bar graph illustrating the proportion of patients in each body mass index (BMI) class with left ventricular outflow tract (LVOT) obstruction (≥30 mm Hg) at rest or with exercise. Increase in BMI is associated with greater likelihood of an obstructive pathophysiology. *p < 0.05 versus normal weight. †p < 0.05 versus pre-obese.

weight range (BMI <25 kg/m^2; average 22.3 ± 2.1), 105 (38%) were pre-obese (BMI 25 to 30 kg/m^2; average 27.4 ± 1.4), and 101 (37%) were obese (BMI >30 kg/m^2; average 35.4 ± 4.8) (Table 1). Overall, 107 patients (39%) were found to have LV outflow obstruction (i.e., a peak instantaneous outflow gradient ≥30 mm Hg) occurring either under basal conditions (n = 57) or during physiologic exercise (n = 50). Seventy-five patients (27%) had a history of controlled systemic hypertension and 14 (5%) had adult-onset, type II diabetes. Left ventricular outflow obstruction, both under resting conditions and elicited by exercise, was disproportionally prevalent in pre-obese and obese patients (Fig. 1, Table 1). Likewise, systemic hypertension and diabetes were more prevalent in pre-obese and obese compared with normal weight patients (Table 1); 38 of the 101 obese HCM patients (38%) were also hypertensive.

Relation of BMI to LV mass, volume, and function. Average LV mass index in the HCM patient cohort was 104 ± 40 g/m^2, ranging from 41 to 329 g/m^2 (highest quartile cutoff 120 g/m^2), greater in males (109 ± 41 g/m^2 vs. 91 ± 36 in females, p < 0.001) (Table 2). Compared with normal weight patients, LV mass index progressively increased in pre-obese and obese patients: 95 ± 46 g/m^2, 100 ± 31 g/m^2, and 114 ± 43 g/m^2, respectively (overall p = 0.005; obese patients p < 0.05 vs. each other group) (Fig. 2), reflecting a direct relationship between LV mass and BMI (correlation coefficient = 0.23; p < 0.001). Thus, obesity in HCM patients was associated with a 120% increase in indexed LV mass, compared with normal body weight. Conversely, maximum LV wall thickness was virtually identical in normal weight, pre-obese, and obese patients (22 ± 6 mm, 21 ± 5 mm, and 21 ± 5 mm, respectively; p = 0.27).

Increased LV mass with respect to body weight was associated with higher LV end-diastolic volume index: 66 ± 14 ml/m^2 in normal weight, 77 ± 18 ml/m^2 in pre-obese, and 83 ± 20 ml/m^2 in obese patients (overall p < 0.001) (Figs. 3 and 4). Therefore, obese patients showed a 126% increase in indexed LV cavity size compared with those of normal weight. Nevertheless, average LV end-diastolic dimension remained within the normal range for each group (i.e., nondilated LV cavity) (Table 2). Notably, when the subset of 168 nonobstructive HCM patients was analyzed separately, the direct correlation of BMI and LV mass index persisted (correlation coefficient 0.22, p = 0.004).

Conversely, LV systolic function, as expressed by ejection fraction, did not differ among the 3 BMI classes (p = 0.86); by virtue of greater end-diastolic volumes, stroke volume index increased from normal weight to pre-obese to obese HCM patients, whereas mass/volume ratio was unchanged (Table 2). Prevalence of LGE was increased in pre-obese and obese HCM patients (48% and 55%, respectively) compared with the normal weight patients (28%, overall p = 0.001). However, average %LV mass occupied by LGE

Table 2 **Cardiovascular Magnetic Imaging Findings in 275 HCM Patients With Respect to BMI**

	Overall	Normal BMI <25 kg/m^2	Pre-Obese BMI 25–30 kg/m^2	Obese BMI >30 kg/m^2	p Value
n	275	69 (25%)	105 (38%)	101 (37%)	
LV end-diastolic volume, ml	151 ± 45	115 ± 29	154 ± 38*	174 ± 45†	<0.001
LV end-systolic volume, ml	43 ± 22	33 ± 17	44 ± 23*	49 ± 25*	<0.001
LV ejection fraction, %	72 ± 10	71 ± 11	72 ± 10	72 ± 9	0.864
Stroke volume, ml	108 ± 33	82 ± 23	109 ± 28*	125 ± 34†	<0.001
Mass/volume ratio	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.4	1.4 ± 0.5	0.428
Patients with LGE	125 (46%)	19 (28%)	50 (48%)*	55 (55%)*	0.001
LGE mass, g	10.4 (6.2–26.6)	14.4 (9.2–50.7)	15.6 (5.6–26.1)	9.5 (4.7–21.2)	0.240
LGE percent of LV volume	2.8 (1.8–6.5)	3.4 (1.8–12.1)	2.9 (1.9–7.3)	2.3 (1.5–5.2)	0.552

Values are n (%), mean ± SD, or median (interquartile range). *p < 0.05 versus normal. †p < 0.05 versus each of the other 2 groups.
LGE = late gadolinium enhancement; other abbreviations as in Table 1.

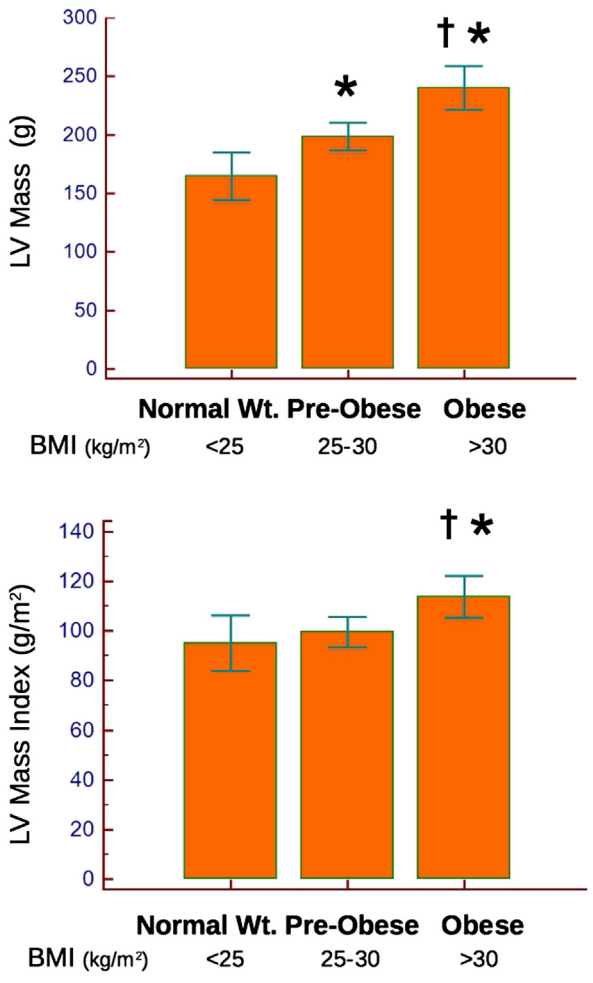

Figure 2 **Impact of BMI on LV Mass**

Each panel shows mean (±95% confidence interval for mean) for unadjusted LV mass and LV mass index in each of the 3 BMI classes. Overall p value for hypertrophic cardiomyopathy patients was 0.005 (obese patients $p < 0.05$ vs. each of the other 2 groups). *$p < 0.05$ versus normal weight; †$p < 0.05$ versus Pre-Obese. Wt = weight; other abbreviations as in Figure 1.

in individual patients did not differ between the subgroups (overall $p = 0.55$) (Table 2).

Systemic hypertension was associated with increased LV mass index in our HCM cohort (118 ± 44 g/m^2 vs. 98 ± 36 g/m^2 in normotensive; $p < 0.001$), although type 2 diabetes was not (LV mass index 104 ± 41 g/m^2 vs. 104 ± 21 g/m^2 in nondiabetic patients; $p = 0.98$). Patients who were both obese and hypertensive had LV mass index values of 126 ± 44 g/m^2, compared with 93 ± 42 g/m^2 in those patients who were neither obese nor hypertensive ($p < 0.001$).

Correlates of LV mass. A multivariate regression model was constructed to identify variables independently associated with greater magnitude of LVH, defined by an LV mass in the highest quartile for the overall cohort, or >120 g/m^2. Variables assessed included BMI, age, sex, resting, or provokable LV outflow obstruction, systemic hypertension, and type 2 diabetes. The model was ultimately constructed with the 3 variables that proved significant at univariate analysis (i.e., obesity, sex, and LV outflow obstruction). In this model, BMI proved an independent correlate of LV mass >120 g/m^2, with a hazard ratio (HR) per unit increase of 1.07 (95% CI: 1.01 to 1.13; $p = 0.019$).

Pre-obese HCM patients showed a 65% increased likelihood of assignment to the highest LV mass index quartile, compared with normal weight patients (HR: 1.65; 95% CI: 0.73 to 3.74; $p = 0.22$), while in obese patients this likelihood increased >300% (HR: 3.1; 95% CI: 1.42 to 6.86; $p = 0.004$). Other variables associated with LV mass >120 g/m^2 were resting or provokable outflow obstruction (HR: 4.9; 95% CI: 2.4 to 9.8; $p < 0.001$), systemic hypertension (HR: 2.2; 95% CI: 1.1 to 4.5; $p = 0.026$), and male sex (HR: 2.1; 95% CI: 0.9 to 4.7; $p = 0.08$).

Symptomatic status and outcome. During a median follow-up of 3.7 years (interquartile range: 2.5 to 5.3 years) following CMR, there were 25 deaths (or equivalents), of which 6 were noncardiac and 19 were HCM-related. Of the latter 19 death events, 12 were sudden (including 7 deaths, 2 patients resuscitated from cardiac arrest, and 3 appropriate implantable cardioverter-defibrillator discharges for ventricular tachycardia/fibrillation). In addition, there were 6 heart failure–related events (3 deaths and 3 heart transplants), and 1 post-operative death (surgical septal myectomy). There was no difference in all-cause mortality among the 3 BMI classes (Fig. 5).

In the 256 patients who were alive at the end of follow-up, those with obesity were however more likely to have developed progressive New York Heart Association (NYHA) functional class III to IV symptoms at most recent evaluation, compared with normal weight patients (overall $p = 0.027$). Noticeably, the proportion of patients in NYHA functional class III at the end of follow-up was 13% among obese patients, compared with 6% among those of normal weight ($p = 0.03$) (Fig. 5). Independent predictors of NYHA functional class III to IV symptoms at end of follow-up were obesity (HR: 3.6; 95% CI: 1.2 to 10.7; $p = 0.02$), female sex (HR: 4.3; 95% CI: 1.5 to 12.4; $p = 0.007$), and LV outflow obstruction (HR: 2.7; 95% CI: 0.9 to 7.8; $p = 0.07$), whereas age, history of atrial fibrillation, and hypertension were not.

Discussion

Obesity and the HCM phenotype. In HCM, the primary morphologic expression of LVH has historically been considered solely a consequence of the gene mutation, with no evidence to date that environmental variables can influence phenotypic expression (2,5,7,8). However, the extreme heterogeneity of phenotypic expression among HCM patients, even in family members sharing the same mutation (6), implies that other determinants of cardiac morphology must be operative (2,3–5). For example, greater LV mass has been observed in male patients and those with dynamic LV

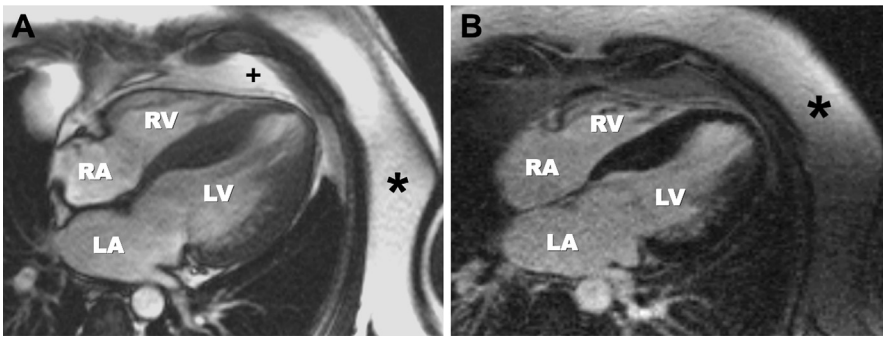

Figure 3 **Cardiac Remodeling in an Obese Patient With Hypertrophic Cardiomyopathy**

Images from a 35-year-old male patient with a body mass index of 28 kg/m^2. Left ventricular mass was 367 g (indexed 153 g/m^2), with a maximal wall thickness of 29 mm. Left ventricular end-diastolic volume was 235 ml (indexed 97 ml/m^2) and left ventricular ejection fraction was 80%. **(A)** Cardiac magnetic resonance steady state free precession 4-chamber showing diffuse thickening with sparing of the apex. **(B)** Corresponding late gadolinium enhancement imaging shows lack of fibrosis in LV. *Subcutaneous fat, $^+$intrathoracic visceral fat. LA = left atrium; LV = left ventricle; RA = right atrium; RV = right ventricle.

outflow obstruction (21), suggesting the disease phenotype may be sensitive to environmental modulation (22). In order to address this issue, we have considered whether obesity, an established cardiovascular risk factor known to promote LVH in the general population, may influence the magnitude of LV mass and prognosis in a multicenter HCM cohort.

Our data demonstrate that obesity is independently associated with increased LV mass, establishing a novel principle that environmental variables can influence disease expression in a primary genetic cardiomyopathy such as HCM, a concept also relevant to other cardiomyopathies (22). Indeed, BMI was powerfully associated with severe LV mass increase in our HCM patients, independent of other important determinants such as sex and dynamic LV outflow obstruction (21), as well as systemic hypertension (18). In our HCM patients, the relationship between BMI and LV mass became particularly evident for BMI values >30. Obese patients were >3 times as likely to have a marked increase in LV mass exceeding 120 g/m^2, compared with those of normal weight. This increase in LV mass was driven primarily by greater end-diastolic volume (which nevertheless remained within normal limits when indexed to body size). In the general population, LV remodeling associated with chamber enlargement is an established consequence of obesity, which normalizes stroke volume index in the presence of increased oxygen requirement, thereby reflecting a physiologic adaptation to body weight (14,15,17,23,24). This principle was also supported by our observation that greater LV cavity volume in obese HCM patients was accompanied by preserved systolic function, resulting in an increased stroke volume index (14,15,23,24). Such impact of obesity on the HCM phenotype is likely mediated by the same triggers producing

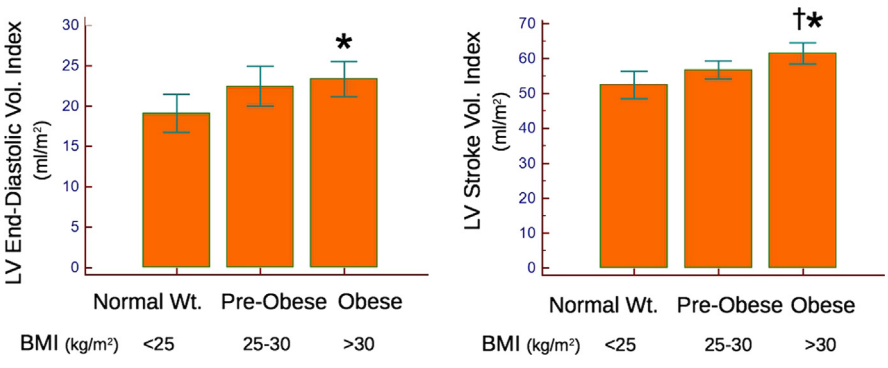

Figure 4 **Impact of BMI on LV Volume and Function**

Panels show mean (±95% confidence interval for mean) LV end-diastolic volume index, LV ejection fraction, and stroke volume index for the 3 BMI classes. Overall p values for each variable are provided in Table 1. *$p < 0.05$ versus normal weight; †$p < 0.05$ versus pre-obese. Wt = weight; other abbreviations as in Figure 1.

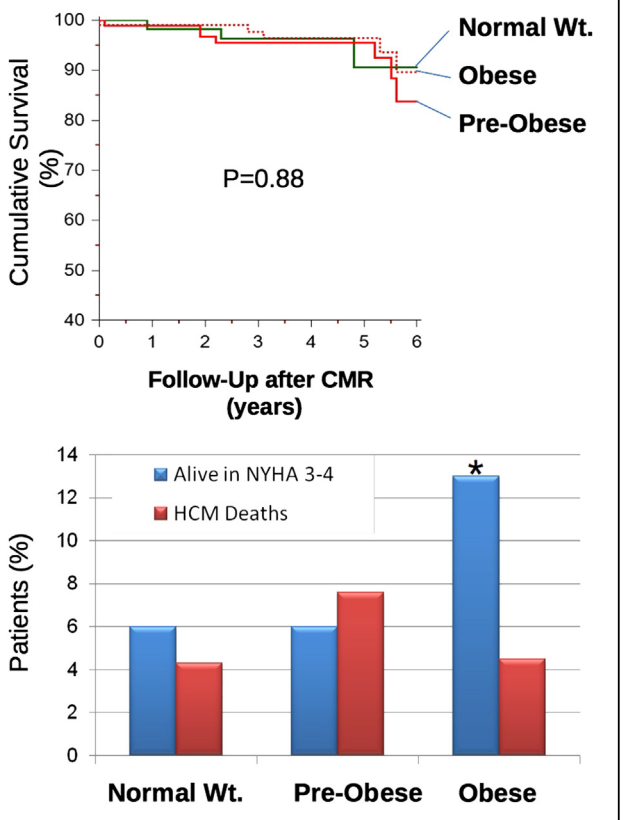

Figure 5 Impact of BMI on Survival

Cumulative risk of all-cause mortality in normal weight, pre-obese, and obese patients during follow-up **(top)**. Prevalence of HCM-related mortality and severe heart failure symptoms (NYHA functional classes III or IV) among survivors at the end of follow-up period **(bottom)**. *p = 0.03 versus other 2 groups. CMR = cardiac magnetic resonance; HCM = hypertrophic cardiomyopathy; NYHA = New York Heart Association; Wt = weight.

increased LV mass in obese individuals without HCM, via the hemodynamic and neurohormonal pathways of secondary hypertrophy (17). The alternative hypothesis that excess body weight may directly act upon the primary etiology of HCM (e.g., by acting as a genetic modifier upon disease-causing sarcomere genes) cannot be excluded but appears much less plausible (22).

Notably, absolute LV wall thickness was unaffected by body weight, with obese patients showing maximum thickness values virtually identical to those in patients of normal weight. This observation suggests that the 2 features, which most differentiate HCM from secondary forms of LVH (i.e., the asymmetric distribution of LV thickening and the often marked degree of regional hypertrophy) are largely unaffected by environmental modulation (4,7). Therefore, neither the current clinical diagnostic criteria for HCM nor decision-making for primary prevention of sudden death with implantable cardioverter-defibrillators (both based on maximum absolute LV wall thickness) (1,3,25–27) require adjustment with respect to BMI in adult patients. In addition, our findings emphasize the importance of assessing total LV mass (by CMR), rather than maximum LV wall thickness, in order to evaluate environmental influences on the HCM phenotype (21). In the present study, for example, mere evaluation of maximum LV thickness in the 3 BMI subgroups would have overlooked the impact of excess body weight on the total hypertrophic burden of our patients.

Obesity and symptomatic status/outcome. During an average follow-up of almost 5 years, obese HCM patients had a 3.6-fold increased risk of developing severe functional limitation (NYHA functional class III/IV) compared with non-obese patients, independent of other known determinants of heart failure symptoms such as outflow obstruction (28) and atrial fibrillation (26). It is impossible to ascertain precisely what proportion of functional limitation was due directly to obesity, as opposed to the consequences of HCM disease state (1,3). Nevertheless, symptomatic obese patients showed no impairment in LV ejection fraction, indicating that their severe disease profile was not due to progressive systolic dysfunction (or "end-stage" HCM) (5). In the general population, obesity is an important predictor of heart failure (12,16,18,29), associated with multiple and often profound changes in the cardiovascular system, including increased cardiac oxygen requirement, sympathetic and neurohormonal activation, increased oxidative stress, increased cardiac output, and expanded central blood volume causing hemodynamic overload in the face of reduced cardiac efficiency (17,29–31). Likewise, our data suggest that excessive body weight in HCM patients may impact importantly on symptom progression, potentially triggering a cycle of events in which obesity leads to an obligatory sedentary lifestyle, further increases in BMI, and, ultimately, worsening of heart failure symptoms (16). Whether significant weight loss will lead to reduction of symptoms and LV mass in obese HCM patients remains unresolved, although these data support future longitudinal studies aimed at clarifying this issue (32).

On the other hand, obesity itself did not confer an independent survival disadvantage during follow-up in our HCM cohort. Although the size of study population and the small number of events does not allow definitive conclusions in this regard, our findings do suggest that other clinical variables may be more relevant than body weight in determining survival in this complex disease (3,5,28), consistent with the elusive relationship of body weight to outcome in cardiovascular disease at large (29,30). Indeed, while obesity is associated with increased morbidity and mortality in the general population (9,13,16,29), a high BMI represents a strong independent predictor of favorable outcome in patients with chronic heart failure, a phenomenon known as the "obesity paradox" (30).

Significance of LV outflow obstruction and hypertension. Left ventricular outflow obstruction was >2-fold more prevalent in obese HCM patients compared with those of normal weight, and associated with a further increase in LV mass (20,21). Although the mechanisms accounting for this

relationship are uncertain, the abnormally increased adrenergic drive associated with obesity may predispose to development of intraventricular gradients (31). Such observations may suggest that excess LVH in obese patients is principally mediated by outflow obstruction and afterload mismatch (21). Nevertheless, when the present analysis was restricted to nonobstructive HCM patients, the association between BMI and LV mass persisted, consistent with the concept that cardiac remodeling due to excess body weight is largely independent of (although synergistic to) outflow obstruction (14,21). We acknowledge that the overall prevalence of LV outflow obstruction in the present cohort (39%) was significantly lower than that reported in a previous study by our group (i.e., 70%) (20). This is largely due to the fact that, in the quoted study, all patients without resting obstruction at baseline were systematically exercised in order to assess provokable obstruction (20), whereas only 105 of 218 such patients were assessed during exercise in the present cohort. Therefore, the true prevalence of obstruction elicited by physiological exercise may have been underestimated in our study.

Systemic hypertension was another modifier of the HCM phenotype (9,13). As expected, the prevalence of elevated blood pressure increased with body weight, and was present in almost 40% of obese patients in our HCM cohort. Even though pharmacologically treated according to existing guidelines (18), hypertension doubled the likelihood of severe LVH in these patients, independent of other determinants of LV mass. Furthermore, the combination of obesity and hypertension was associated with the highest LV mass values observed for any subset within the cohort. Thus, the present findings support the concept that the neurohormonal abnormalities associated with hypertension may impact LV mass in HCM patients (16,23,33), and thereby represent a relevant therapeutic target (16,18). To this regard, it is important to acknowledge that environmental influences of single cardiovascular risk factors on the HCM phenotype are unavoidably linked to a number of coexisting factors (22). For example, obesity is per se associated with an increased likelihood of hypertension, diabetes, and dynamic LV outflow obstruction (11,16). Therefore, interpretation of the clinical role of each of these factors, in isolation from the overall pathophysiological picture, remains challenging.

Conclusions

The present study provides evidence that obesity is independently associated with adverse cardiac remodeling and increase in LV mass in HCM patients. These observations underscore the novel principle that the primary phenotypic expression in this complex, heterogenous heart disease is also subject to environmental variables and not solely the product of disease-causing sarcomere mutations. In addition, obesity appears to play a role in the development and progression of heart failure symptoms in HCM, supporting the need for follow-up studies clarifying whether modulating obesity can improve clinical course.

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Key Words: cardiac magnetic resonance ■ hypertrophic cardiomyopathy ■ hypertrophy ■ obesity ■ outcome.

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EDITORIAL COMMENT

Obesity and Hypertrophic Cardiomyopathy

Chickens, Eggs, and Causality: Clinical Skills Remain the Key to Caring for Patients*

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Francisco Lopez-Jimenez, MD, MSc
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The impact of obesity on the cardiovascular system continues to reveal itself. In this issue, Olivotto et al. (1) share the results of a 3-center collaboration on body mass index and its relation to left ventricular morphology in patients with hypertrophic cardiomyopathy (HCM). The investigators found that obese HCM patients had significantly larger left ventricular mass, left ventricular mass index, and higher subsequent rates of symptom development. These results suggest that environmental/situational factors can influence ventricular morphology in a condition that is genetically inherited. Notably, only 25% of the patients in this study fell

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into the normal weight classification. So, like the age-old question, can we tell which came first? Is it a more severe expression of HCM that leads to obesity, or is it the general trend of increasing body weight in Western society that complicates HCM?

Because this was an observational study, we cannot tell the direction of the association. There are numerous potential pathophysiologic mechanisms by which obesity could result in more hypertrophy. These include increased sympathetic tone, increased leptin levels, myocardial fatty infiltration, insulin resistance, and enhanced renin-angiotensin activity (2). Likewise, a more severe phenotypic expression of HCM could readily lead to a less active life with all the attendant negative impacts, including increasing body mass. While at baseline, the study subjects had statistically similar symptom status; there is no indication of daily activity levels, nor objective measures of exercise capacity. In the end, treating patients with HCM and obesity will necessarily involve treating both aspects.

The impact of obesity on symptom status is a troubling clinical conundrum. Obesity is a well-known cause of heart failure, and has been shown to result in worsening diastolic function independent of other comorbid conditions (3,4). Patients with obesity have higher overall oxygen requirements as well as abnormal myocardial function as evidenced by strain and strain rate imaging (5). In the Olivotto et al. study, the patients with obesity were more likely to have left ventricular outflow tract obstruction, and to be receiving diuretics, and/or pure vasodilators as part of their therapy. Those medications are well known to exacerbate left ventricular outflow tract obstruction.

Why is this a problem in patients with HCM? Other than defibrillators for prevention of sudden cardiac death, all therapies in HCM have the sole indication of symptom relief (6). The therapies employed, including medications and invasive procedures, can be quite effective when combating symptoms due to hemodynamic derangements, but not likely to be effective at when directed at problems such as deconditioning and excess oxygen requirements. Similarly, the need to consider surgical or invasive therapy is based on persistent symptoms that are unresponsive to pharmacologic therapy. However, if the symptoms are persistent because they are primarily related to the obesity, then the medications (and the subsequent procedures) have little chance of making an impact on symptoms.

So, when facing an obese patient with HCM and deciding whether to start, increase, or decrease therapy one has to make important considerations of the balance between hemodynamics and excess body weight. By the time such patients have become highly symptomatic, the combination of sedentary status, deconditioning, and increased tendency to outflow tract obstruction may make therapeutic lifestyle change untenable. Yet, treating the hemodynamic abnormalities will lead to less than satisfying results unless weight loss and healthy habits are taught to and adopted by the patients.

How does one then approach this situation? With cardiopulmonary exercise testing we can determine whether patients exercise capacity is limited by cardiac output limitations based on a plateau in the peak oxygen consumption (VO_2). Conversely, if a patient is purely limited due to deconditioning and obesity, then the peak oxygen consumption (VO_2) will continue to rise through the point at which the patient has to stop exercising. Armed with this information, appropriate therapeutic decisions and focused counseling can help our patients understand the expected impact, and their role in achieving a better quality of life. Even with completely successful treatment of hemodynamic considerations, the obese patient needs to understand this is the first step in their path to improved functional capacity. Long-term exercise and dietary modifications will need to continue. Importantly, therapeutic lifestyle change,

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particularly if the cardiac hemodynamic abnormalities are mild, may represent the most important step to recovery. Whether purposeful weight loss in symptomatic patients with HCM improves left ventricular morphology and hemodynamics is yet to be determined.

The study by Olivotto et al. (1) suggested that some of these findings were independent of comorbid conditions. However, as clinicians we must not discount the increased rates of hypertension, diabetes, coronary artery disease, and sleep apnea have important roles not only in the cardiovascular system but in general well-being. Furthermore, each of these conditions has been shown to be associated with adverse outcomes in patients with HCM. Treating and caring for patients with HCM means not just understanding the pathophysiology of diastole and outflow tract obstruction, but must incorporate treating comorbid conditions aggressively.

Another of the management considerations for patients with HCM deserves mention. Many patients perceive, or are in fact inappropriately counseled by their physicians, that they should not engage in regular exercise. This stems from the fact that sudden cardiac death among competitive athletes is most often related to HCM such that competitive athletics are discouraged for these patients. To extend that discouragement to regular, noncompetitive exercise is a travesty. Counseling and encouraging healthy habits, including mild to moderate intensity exercise, is an important aspect of caring for patients.

Hypertrophic cardiomyopathy is generally well tolerated, with a good prognosis, and completely compatible with a normal lifestyle (often with no therapeutic interventions). That obesity modifies the ventricular morphology, and the overall well-being of our patients, provides further evidence that we need to actively teach our patients about healthy choices. Which comes first? Maybe we will find out one day, but for now it is our responsibility to treat both. Clinically, we treat people, not just pathophysiology.

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Key Words: body mass index ■ hypertrophic cardiomyopathy ■ left ventricular hypertrophy ■ obesity ■ outcome.

Improving Survival Rates of Patients With Idiopathic Dilated Cardiomyopathy in Tuscany Over 3 Decades: Impact of Evidence-Based Management

Gabriele Castelli, Alessandra Fornaro, Mauro Ciaccheri, Alberto Dolara, Vito Troiani, Benedetta Tomberli, Iacopo Olivotto and Gian Franco Gensini

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Improving Survival Rates of Patients With Idiopathic Dilated Cardiomyopathy in Tuscany Over 3 Decades

Impact of Evidence-Based Management

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Background—Contemporary therapeutic options have led to substantial improvement in survival of patients with heart failure. However, limited evidence is available specifically on idiopathic dilated cardiomyopathy. We thus examined changes in prognosis of a large idiopathic dilated cardiomyopathy cohort systematically followed during the past 30 years.

Methods and Results—From 1977 to 2011, 603 consecutive patients (age, 53±12 years; 73% men; left ventricular ejection fraction, 32±10%) fulfilling World Health Organization criteria for idiopathic dilated cardiomyopathy, including negative coronary angiography, were followed up for 8.8±6.3 years. Patients were subdivided in 4 enrollment periods on the basis of heart failure treatment eras: (1) 1977–1984 (n=66); (2) 1985–1990 (n=102); (3) 1991–2000 (n=197); (4) 2001–2011 (n=238). Rates of patients receiving angiotensin-converting enzyme inhibitors/angiotensin receptors blockers, β-blockers, and devices at final evaluation increased from 56%, 12%, 8% (period 1) to 97%, 86%, 17% (period 4), respectively (P<0.05). There was a trend toward enrollment of older patients with less severe left ventricular dilatation and dysfunction during the years. During follow-up, 271 patients (45%) reached a combined end point including death (heart failure related, n=142; sudden death, n=71; and noncardiac, n=22) or cardiac transplant (n=36). A more recent enrollment period represented the most powerful independent predictor of favorable outcome {period 2 versus 1 (hazard ratio [HR], 0.64; P=0.04), period 3 versus 1 (HR, 0.35; P<0.001), period 4 versus 1 (HR, 0.14; P<001)}. Each period was associated with a 42% risk reduction versus the previous one (HR, 0.58; 95% confidence interval, 0.50–0.67; P<0.001), reflecting marked decreases in heart failure–related mortality and sudden death (period 4 versus 1: HR, 0.10; P<001 and HR, 0.13; P<0.0001, respectively).

Conclusions—Evidence-based treatment has led to dramatic improvement in the prognosis of idiopathic dilated cardiomyopathy during the past 3 decades. The benefits of controlled randomized trials can be replicated in the real world, emphasizing the importance of tailored follow-up and long-term continuity of care. **(*Circ Heart Fail*. 2013;6:913-921.)**

Key Words: cardiac resynchronization therapy ■ cardiomyopathy, dilated ■ drug therapy ■ heart failure ■ outcomes assessment

Idiopathic dilated cardiomyopathy (IDCM) is a severe myocardial disease characterized by dilatation and impaired function of the left or both ventricles,[1] affecting >36.5 individuals per 100 000.[2] IDCM accounts for ≈50 000 hospitalizations and 10 000 deaths each year and is responsible for ≈25% of all cases of heart failure (HF) in the United States.[3] During the past 3 decades, the outcome of IDCM is presumed to have radically changed after major advances in pharmacological and device-based therapeutic strategies for HF; however, studies addressing the outcome of HF have been characterized by relatively small representation of individuals with IDCM, so that limited data exist with specific regard to this condition.[4–11]

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In addition, real-world implementation of standard HF guidelines is challenging, with rates of compliance that can be considerably lower than those reported in clinical trials documenting survival benefits.[12–17] As a result, the issue of long-term outcome of IDCM in the community remains largely unresolved. In the present study, we therefore chose to examine the clinical features and prognosis of a sizeable cohort of unselected, consecutively enrolled and angiographically negative patients with IDCM from a well-defined regional population in Tuscany, evaluated during the past 30 years in a systematic fashion and by the same team, in relation to the evolving treatment strategies including evidence-based pharmacological and device-based interventions.

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Methods

Setting and Study Population

The Referral Center for Cardiomyopathies has been established in the mid-1970s in Careggi University Hospital, a large community-based multispecialty hospital in Florence, Italy. Careggi is a tertiary center with >1500 beds, serving the Florence metropolitan area (population ≈1 million) and the surrounding geographic region of Tuscany (total population ≈3 700 000 within ≈23 000 km^2).[18] In this setting, between January 1977 and September 2011, we consecutively enrolled 1085 patients with a diagnosis of dilated cardiomyopathy. In 271 patients, the condition was judged to be secondary to ischemic heart disease, systemic hypertension, chemotherapy, alcoholic abuse, diabetes mellitus, cor pulmonalis, valve disease, or other cardiac or systemic diseases. In addition, 211 patients in whom a coronary angiogram was not available were excluded. The remaining 603 were classified as IDCM, according to the World Health Organization criteria and in the presence of a negative coronary angiogram: these comprise the present study cohort.

Evaluation and Follow-Up

All study patients were evaluated and followed up at our institution by clinical and family history, physical examination, 12-lead ECG, standard chest radiograph, routine laboratory tests, 24-hour Holter ECG monitoring (since early 1980s), M-mode and 2D echocardiography (since 1970s), and Doppler echocardiography (since mid-1980s). Exercise stress test and cardiac catheterization were performed as dictated by clinical requirements. Endomyocardial biopsy has been performed routinely until the early 1990s and thereafter only on selected patients with suspect active myocarditis. Although the family history of each patient was investigated, systematic family screenings have not been performed. In 51 patients (8%) a genetic transmission of IDCM was evident or suspected by virtue of clinical and echocardiographic documentation of the disease in a relative or by a family history of HF-related death or premature sudden death (SD).

Patients were regularly followed up by outpatient visits every 6 months (or more frequently when clinically indicated) and, when necessary, by interviews with referring physicians or by telephone contacts. For the purposes of this study, follow-up ended on September 30th, 2011. In patients who died or were transplanted, end of follow-up was considered as time of death or heart transplantation. In the minority of patients lost to follow-up (ie, not traceable by September 30th, 2011), the last clinical evaluation of telephone contact was considered. In the past 5 years of the study (2006–2011), the proportion of patients who were actively followed up, had died/transplanted, or were lost in period was 44%, 45%, and 11%, respectively. For the entire study period, patients were seen by the same cardiologists who assumed primary responsibility for management. The use of pharmacological agents, implantable cardioverter defibrillator (ICD), and biventricular pacing for cardiac resynchronization therapy (CRT) was carefully considered as these became available and implemented when felt appropriate, according to existing guidelines.[19] Pharmacological treatment was carefully titered to achieve maximum tolerated doses of angiotensin-converting enzyme inhibitors/angiotensin receptors blockers (ACEI/ARBs) and β-blockers. Candidates for heart transplantation (HTx) were assessed jointly with the Regional Heart Transplant Referral Center in Siena, where the operations were performed. The study was approved by the institutional review committee and patients gave an informed consent.

Enrollment Periods

To assess long-term changes in outcome in relation to treatment options, we subdivided our patients with IDCM into 4 periods, coinciding with different therapeutic eras of HF treatment: period 1: 66 patients (11%) enrolled from 1977 to 1984, defined as the pre-ACE inhibition era, when standard therapy consisted of diuretic agents, digoxin, and early vasodilators; period 2: 102 patients (17%) enrolled from 1985 to 1990, marking the beginning of the ACEI era; period 3: 197 patients (33%) enrolled from 1991 to 2000, characterized by increasing use of ACEI and ARBs and the introduction of β-blockers; and period 4: 238 patients (39%) enrolled from 2001 to 2011, the device-era, characterized by the introduction of ICD and CRT on top of extensive neurohormonal blockade.[20]

Statistical Analysis

Student *t* test and 1-way ANOVA were used to compare continuous variables, whereas categorical variables were compared by χ^2 test or Fisher method, as appropriate. Univariate survival estimates were obtained using Kaplan–Meier method. Forward conditional Cox proportional hazards regression analysis was used to evaluate the relationship between periods of enrollment, clinical and instrumental baseline data of patients, and long-term outcome for the following end points: (1) death from any cause including appropriate ICD interventions and HTx; (2) SD; (3) death because of refractory HF. The following variables were included in the models: age, New York Heart Association (NYHA) class, sex, left ventricular ejection fraction (LVEF), baseline LV end-diastolic diameter index, left atrial diameter index, and the presence of moderate to severe mitral regurgitation at enrollment. A P value <0.05 was considered statistically significant. Statistical analysis was performed with the SPSS package, version 20 (SPSS Inc, Chicago, IL).

Results

Baseline Clinical Features

Mean age of the 603 patients at first evaluation was 53±12 (range, 16–75) years; 442 (73%) were men. In 51 patients (8%) there was a family history of IDCM. Average NYHA functional class was 2.3±0.8; 82 patients (14%) were in class I, 265 (44%) in class II, 198 (33%), and 58 (10%) in class III or IV. Mean end-diastolic diameter index was 36±6 mm/m^2, LVEF was 31±10%, and left atrial diameter index was 24±4 mm/m^2; moderate to severe mitral regurgitation was diagnosed in 106 patients (18%). One hundred seventeen patients (19%) had ECG evidence of left bundle-branch block, whereas a history of paroxysmal or permanent atrial fibrillation was recorded in 123 (21%). Of the 531 patients with 24-hour ambulatory Holter monitoring, 183 (30%) showed non–sustained ventricular tachycardias ≥3 beats and sustained ventricular tachycardias (Table 1).

Comparison of Enrollment Periods

At initial evaluation, the 4 groups of patients identified based on the period of enrollment were comparable with regard to sex and NYHA class (Table 1). However, there was a slight trend toward enrollment of older patients with less severe LV dilatation and dysfunction during the years: in period 4 versus period 1, age was 55±12 versus 50±11 years, respectively (P<0.0001); end-diastolic diameter index was 34±5 versus 39±6 mm/m^2 (P<0.0001), and LVEF was 33±9 versus 29±11% (P=0.016). The overall prevalence of atrial fibrillation was similar in the 4 periods; however, permanent atrial fibrillation at initial diagnosis became less prevalent over time (overall P<0.001; Table 1).

Evolution in Management

Expectedly, medical treatment at enrollment differed significantly between the 4 patient periods (Table 2; Figure 1). ACEI and ARBs were extensively used at our institution since early 1990s, reaching a 96% rate at enrollment after 2001 versus 4% before 1985 (P<0.0001). The use of β-blockers increased significantly after 2001: 79% of patients were already treated with these agents at enrollment in period 4 compared with 0%

Table 1. Baseline Clinical and Instrumental Characteristics of Patients at Enrollment

	Total	Period 1 (1977–1984) n=66	Period 2 (1985–1990) n=102	Period 3 (1991–2000) n=197	Period 4 (2001–2011) n=238	*P* Value
Men, %	442 (73%)	50 (76%)	75 (73%)	149 (76%)	168 (71%)	NS
BSA, kg/m^2	1.9±0.2	1.8±0.2	1.8±0.2	1.8±0.2	1.9±0.2	<0.001
Age, y	53±12	50±11	48±13	54±12	55±12	<0.001
Follow-up, mo	106±75	113±108	135±99	122±64	79±47	<0.001
Familial IDCM, %	51 (8%)	1 (1%)	2 (2%)	22 (11%)	26 (8%)	0.003
LBBB, %	117 (19%)	19 (29%)	32 (31%)	35 (18%)	31 (13%)	NS
Paroxysmal AF	29 (5%)	0 (0%)	2 (2%)	8 (4%)	19 (8%)	<0.001
Chronic AF	94 (16%)	18 (27%)	21 (21%)	29 (15%)	26 (11%)	<0.001
Complex VA	183 (30%)	27 (41%)	46 (45%)	65 (33%)	45 (19%)	<0.001
NYHA class I	82 (14%)	6 (9%)	10 (10%)	31 (16%)	35 (15%)	NS
NYHA class II	265 (44%)	33 (50%)	50 (49%)	89 (45%)	93 (39%)	
NYHA class III	198 (33%)	21 (32%)	31 (30%)	63 (32%)	83 (35%)	
NYHA class IV	58 (10%)	6 (9%)	11 (11%)	14 (7%)	27 (11%)	
EDD, mm	67±8	69±8	69±9	67±9	65±8	<0.001
iEDD, mm/m^2	36±6	39±6	38±6	36±5	34±5	<0.001
LVEF, %	31±10	29±11	30±10	31±9	33±9	0.016
LVFS, %	18±6	16±5	17±6	19±6	19±6	0.015
ARD, mm	33±4	33±4	32±4	32±4	33±4	0.022
iARD, mm/m^2	17±3	17±5	18±3	17±2	17±3	NS
LAD, mm	44±7	45±7	46±8	44±7	44±7	NS
iLAD, mm/m^2	24±4	25±4	25±5	24±4	23±4	<0.001
Moderate to severe MR	106 (18%)	3 (4%)	12 (12%)	29 (15%)	62 (26%)	<0.001

Data are presented as mean±SD or percentages. AF indicates atrial fibrillation; ARD, aortic root diameter; BSA, body surface index; EDD, end-diastolic diameter; iARD, aortic root diameter index; IDCM, idiopathic dilated cardiomyopathy; iEDD end-diastolic diameter index; iLAD, left atrial diameter index; LAD, left atrial diameter; LBBB, left bundle-branch block; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; MR, mitral regurgitation; NS, not significant; NYHA, New York Heart Association; and VA, ventricular arrhythmias.

in period 1, 1% in period 2, and 21% in period 3 (P<0.0001). These differences were still evident at the end of follow-up, although the rate of treated patients considerably increased in all patient subgroups, reflecting changes in management over time (Table 2). Of note, 97% and 86% of patients in period 4 were on ACEI/ARBs and β-blockers, respectively, at final evaluation. The use of mineralocorticoid receptor antagonists also increased progressively in periods 1 to 4, whereas that of digoxin decreased (at final evaluation, only 37% of patients in period 4 were on digoxin versus 92% in period 1; P<0.0001). The ICD and CRT were introduced in 1998 and 2000, respectively, and their use increased steadily over time (Table 2). Overall, 79 patients (13%) were implanted with an ICD, whereas 69 (11%) received combined ICD/CRT (Figure 1).

Table 2. Treatment at Enrollment and at the End of Follow-Up

	Total		Period 1 (1977–1984) n=66		Period 2 (1985–1990) n=102		Period 3 (1991–2000) n=197		Period 4 (2001–2011) n=238		*P* Value	
	Enrollment	End F-Up	Enrollment	End F-Up	Enrollment	End F-Up	Enrollment	End F-Up	Enrollment	End F-Up	Enrollment	End F-Up
ACEI/ARBs	467 (77%)	552 (91%)	3 (4%)	37 (56%)	53 (52%)	93 (91%)	182 (92%)	192 (97%)	229 (96%)	230 (97%)	<0.001	<0.001
β-Blockers	232 (38%)	344 (57%)	0 (0%)	8 (12%)	1 (1%)	22 (22%)	42 (21%)	110 (56%)	189 (79%)	204 (86%)	<0.001	<0.001
Digoxin	338 (56%)	359 (59%)	60 (91%)	61 (92%)	80 (78%)	83 (81%)	117 (59%)	127 (64%)	81 (34%)	88 (37%)	<0.001	<0.001
Diuretics	483 (80%)	502 (83%)	61 (92%)	63 (95%)	87 (85%)	91 (89%)	153 (78%)	164 (83%)	182 (76%)	184 (77%)	0.01	0.001
MRA	155 (26%)	243 (40%)	7 (11%)	24 (36%)	12 (12%)	39 (38%)	41 (21%)	77 (39%)	95 (40%)	103 (43%)	<0.001	<0.001
Vasodilators	58 (10%)	96 (16%)	8 (12%)	20 (30%)	5 (5%)	13 (13%)	22 (11%)	37 (19%)	23 (10%)	26 (11%)	NS	0.001
Warfarin	131 (22%)	203 (34%)	6 (9%)	27 (41%)	20 (20%)	44 (43%)	43 (22%)	56 (28%)	62 (26%)	76 (32%)	0.03	0.04
Amiodarone	103 (17%)	155 (26%)	10 (15%)	26 (39%)	20 (20%)	36 (35%)	33 (17%)	44 (22%)	40 (17%)	49 (21%)	NS	0.001
ICD	…	79 (13%)	…	5 (8%)	…	9 (9%)	…	24 (12%)	…	41 (17%)	…	NS
CRT	…	69 (11%)	…	3 (4%)	…	7 (7%)	…	18 (9%)	…	41 (17%)	…	0.003

ACEI indicates angiotensin-converting enzyme inhibitors, ARBs, angiotensin receptor blockers; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter defibrillator; MRA, mineralocorticoid receptor antagonists; and NS, not significant.

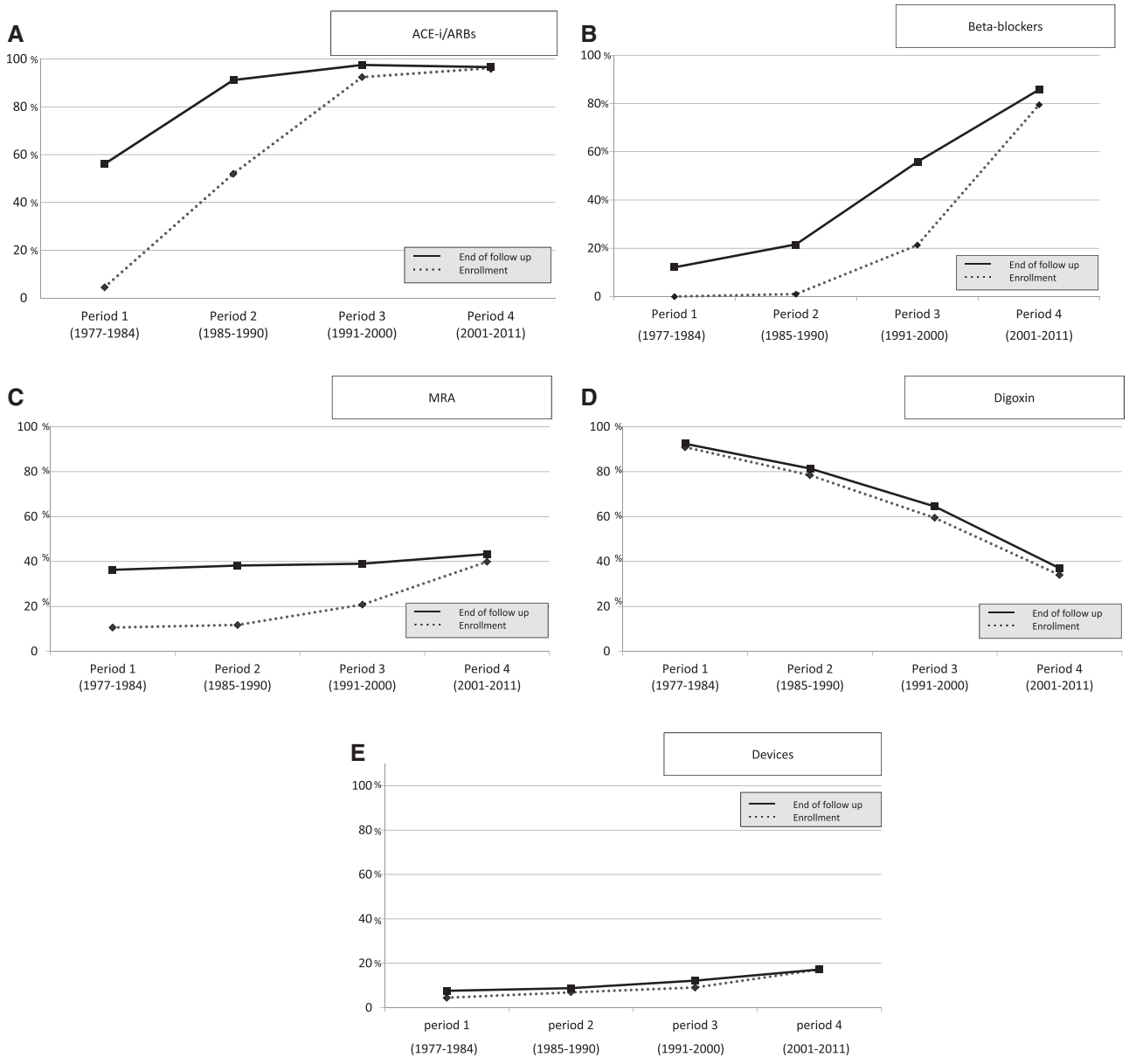

Figure 1. Evolution in pharmacological and device therapy from initial evaluation to end of follow-up based on enrollment period. **A** to **D**, The dotted line indicates treatment rates at enrollment for each period, whereas the straight line indicates treatment rates at final evaluation. **E**, The dotted line indicates implant rates at enrollment for each period, whereas the straight line indicates implant rates at final evaluation. ACEI/ARB indicates angiotensin-converting enzyme inhibitor/angiotensin receptor blockers; and MRA, mineralocorticoid receptor antagonists.

Notably, 52% of patients that received an ICD and 59% of those with CRT were enrolled in period 4; the remaining were implanted in patients enrolled in previous periods, who were still actively followed up when the devices became available.

Changes in Functional Status and Reverse Remodeling

On average, patients enrolled in periods 3 and 4 improved their functional status during follow-up, whereas those enrolled earlier showed symptomatic progression (Figure 2). Improvement in systolic LV function compared with baseline was observed in all groups, although most evident in periods 3 and 4. Likewise, a reduction in end-diastolic diameter index occurred to a greater extent in those patients enrolled most recently (Figure 2). This was paralleled by a less common occurrence of worsening mitral regurgitation after 1990 (33% in period 1; 37% in period 2; 28% in period 3; and 11% in period 4; overall $P<0.0001$).

Long-Term Outcome and Predictors of Risk

During an average follow-up of 8.8±6.3 years, 271 patients (45%) reached the combined end point including all-cause mortality and HTx. Of these, 142 patients (23%) died because of refractory HF, 71 (12%) because of SD, 22 (4%) died of noncardiac causes, and 36 patients (6%) underwent HTx. Overall survival was 79% and 63% at 5 and 10 years, respectively. There was marked and progressive improvement in outcome from periods 1 to 4 for all end points (Figure 3).