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CASE REPORT

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The left and right ventricle of a patient with a R723G mutation of the beta-myosin heavy chain and severe hypertrophic cardiomyopathy show no differences in the expression of myosin mRNA

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Abstract

Background: *In familial hypertrophic cardiomyopathy (FHC), asymmetric left ventricular (LV) hypertrophy has been considered to be the predominant phenotypic expression, whereas right ventricular (RV) involvement is still ambiguous. In most cases, the right ventricle remains unaffected until secondary pulmonary hypertension develops. Several FHC-causing mutations of genes encoding sarcomere-related proteins have been identified which are transmitted in an autosomal-dominant manner.*

Methods: *We report the case of a 61 year old member of a Catalan family with a Arg723Gly missense mutation of the β-myosin heavy chain (β-MHC), that is associated with a malignant phenotype characterized by sudden cardiac death and heart failure. Because of progressive systolic LV dysfunction, the patient received a heart transplant in 2003.*

Results: *Molecular analysis of the myocardial tissue of the explanted heart, taken from the left and right ventricle, showed a similar deviation of the ratio of mutant* vs *wild type mRNA of the β-MHC of 71.8 ± 5% and 68.5 ± 3%, respectively. This finding was confirmed for LV biopsies of this patient on protein level, showing a similar proportion of mutated β-myosin. But since the patient is heterozygous for the β-MHC mutation and the mutation is located in a coding region, the relative increase of the expression of the mutant allele is unexpected. It has been demonstrated before by our group for several β-MHC mutations that the relative abundance of mutated mRNA/ /protein correlates with the clinical severity of the disease. But since the right ventricle shows no (or only minor) manifestation in terms of hypertrophy or dysfunction, the level of mRNA and protein expression is not the only factor responsible for the development of the phenotype of FHC.*

Conclusions: *Several mechanisms through which cardiac stresses may incite maladaptive cardiac remodeling primarily of the left ventricle that result in myocardial hypertrophy and heart failure are proposed. One of those triggers could be the enhanced work load of the left ventricle, especially if a LV outflow tract gradient is present, in contrast to the lesser demands to the right ventricle which is adapted to the low pressure system of the pulmonary circulation. Further studies are needed to confirm the results of this case, as well as functional studies involving both ventricles.* (Cardiol J 2010; 17, 5: 518–522)

Key words: hyperthropic cardiomyopathy, molecular biology, hypertrophy

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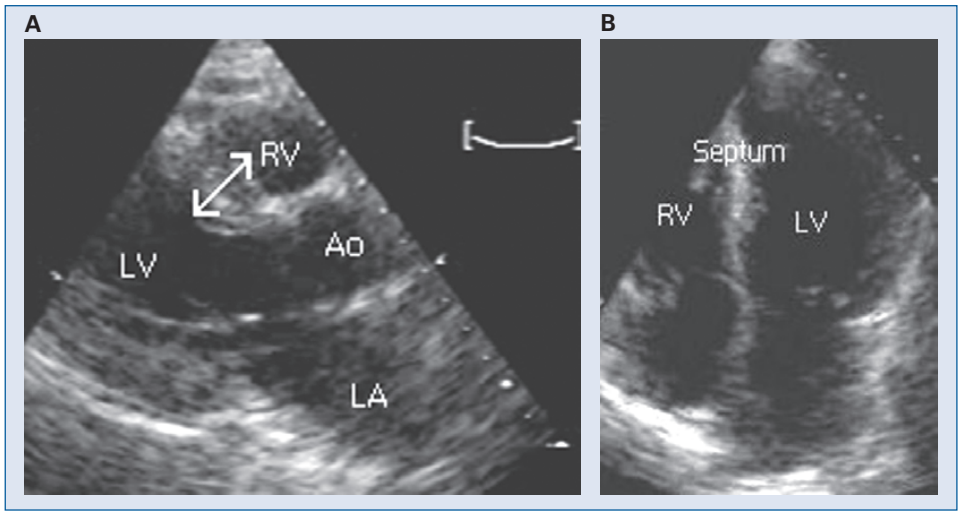

Figure 1. A. Six years prior to heart transplantation (1997), in the parasternal long axis view, assymmetric septal hypertrophy (septum diameter 16 mm, see double arrow, posterior wall 13 mm), and a moderately increased left ventricular cavity size (end-diastolic diameter 57 mm) was recorded. The function and size of the right ventricle was normal; **B.** In the four chamber view, the interpretation of the recording concerning the right ventricle is limited, but it was documented as non-dilated; Ao — aortic root, LA — left atrium; LV — left ventricular cavity; RV — right ventricular chamber.

Introduction

Familial hypertrophic cardiomyopathy (FHC) is the commonest inherited cardiac disease that is characterized by left ventricular (LV) hypertrophy, predominantly of the interventricular septum. The clinical spectrum of the disease varies greatly, ranging from asymptomatic mutation carriers to a small subset of patients who develop progressive systolic heart failure and LV dilatation, with a reported prevalence of 2.4% to 15% in different series [1]. In about one third of genetically characterized FHC families, point mutations of the β-MHC gene were shown to cause the disease [2]. In 2000, Enjuto et al. [3] reported on a new Arg723Gly missense mutation in the converter region of the β-MHC in three Catalan families. This mutation was associated with sudden death and end-stage heart failure. In one of these families, nine gene mutation carriers were found, all showing distinct morphologic manifestations of the disease in the left ventricle on echocardiography (ECHO). To address the interesting phenomenon that the phenotype of FHC is predominantly found in the left ventricle, we analysed the ratio of mutant *versus* wild type β-MHC mRNA in myocardial tissue of the left and right ventricles of a member of one of the Catalan families. This patient had initially developed the classical clinical phenotype of FHC with asymmetrical septal hypertrophy and diastolic dysfunction, which then further progressed into LV dilatation and systolic heart failure. Consequently, the patient received a heart transplant aged 55.

Methods

The patient, who is now aged 61, of the genotyped Catalan family 26 (patient II-5, Enjuto et al.), a heterozygous carrier of a point mutation (Arg723Gly) of the β-MHC, was diagnosed with FHC at the age of 31. He developed the classical spectrum of symptoms of FHC over subsequent years, such as atypical chest pain, dyspnea (NYHA II) and palpitations. Pharmacological treatment was started with a moderate dosage of the calcium channel blocker verapamil (120 mg to 240 mg per day).

In 1997, he suffered a syncopal episode with runs of sustained ventricular tachycardia, based on which he received an automatic implantable defibrillator. During that time, a comprehensive cardiologic study was performed showing on ECHO an asymmetric LV hypertrophy with a maximum thickness of the septum of 16 mm and of 13 mm of the posterior wall in the parasternal long axis (Fig. 1A). The ejection fraction (EF) was moderately decreased to 40% and the LV end-diastolic diameter was slightly increased to 57 mm. The mitral deceleration time of 265 ms was indicative of diastolic dysfunction that was confirmed angiographically with an end-diastolic pressure in the left ventricle of 20 mm Hg. The coronary arteries were normal. The right ventricle

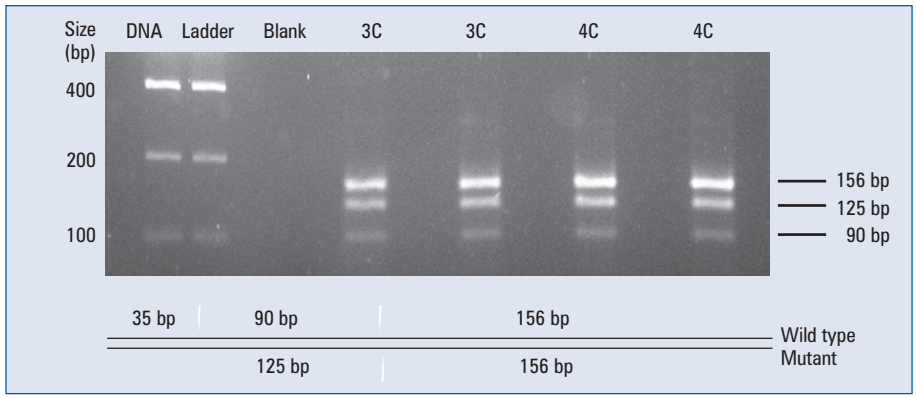

Figure 2. On the gel electrophoresis of the digested PCR products (3.5% ethidium bromide agarose gel), the uppermost 156 base pair (bp) band is derived from the mutant and wild type, the 125 bp exclusively from the mutant and the 90 bp exclusively from the wild type. The 35 bp fragment, also produced by the digestion of the wild type PCR product, is not visible owing to its small size (see cartoon below). The cycle numbers (C) are given on top of each lane (two times 3C and 4C).

showed no obvious manifestation of the disease on ECHO at that time (Fig. 1A, B).

From that point on, a slow but progressive deterioration of the functional status of the patient developed, which became severe (NYHA III–IV) after the commencement of repeated episodes of atrial fibrillation in 2001. On at least two occasions, he suffered acute LV failure with pulmonary oedema, and he persisted in severe functional limitation despite good heart rate control. During that time, he was treated with furosemide (40–80 mg per day), amiodarone, digoxine, low dosages of enalapril, and oral anticoagulation following a TIA (transient cerebral ischemic attack) in January 2002. He did not tolerate beta-blocker therapy because of severe low output. Echocardiographic data from May 2002 showed an ejection fraction that had declined to 30%, an LV end-diastolic diameter of 65 mm with less prominent septal hypertrophy with 13 mm, and a mitral diastolic flow showing a restrictive filling pattern of the left ventricle. The invasive hemodynamic data displayed the clinical picture of progressive low output syndrome and LV backward failure with a mean pulmonary artery pressure of 64/24 (40) mm Hg. He was promptly scheduled for heart transplantation and successfully operated upon in September 2003. He has done well since then, despite some extracardial problems like advanced reflux esophagitis, and prostate cancer that is currently in full remission after radiotherapy.

The study was approved by the local bioethical committe and all patients gave their informed consent.

Total RNA of each of the nine tissue samples frozen in liquid nitrogen were isolated by using the cells to cDNA kit II (Applied Biosystems, USA). Isolated RNA was reverse transcribed with a common gene specific primer (5' TGC CAG GTT GTC TTG TTC CG 3'). For cDNA amplification two gene specific primers ([forward primer 5' CCA ACC GCA TCC TCT ACG GGG ACT TCC GGC AGA GGG AT 3'] and [reverse primer 5' CTT TTT GTA CTC CAT TCT GGC GAG CAC A 3']) were used. The PCR products were digested with NdeII restriction enzyme for discriminating between the wild type and the mutated cDNA, which was possible because the forward primer created an additional restriction site only in the wild type PCR product. The fragments were separated on a 3.5% agarose gel after ethidium bromide staining. For relative quantification, the intensity profiles of the individual bands and of an equimolar DNA-standard were obtained densitometrically. The fraction of mutated mRNA in each restriction digest was calculated from the IOD/bp ratio of a fragment exclusively from the mutant versus the IOD/bp ratio of a fragment exclusively from the wild type or versus the average of the IOD/bp values of two wild type fragments (Fig. 2). For each biopsy, 6 ± 2 (mean ± SD) lanes were analyzed.

Results

Myocardial tissue from a 61 year-old patient (II-5, Enjuto et al.) of a genotyped Catalan family was analyzed. The clinical disease spectrum of the nine mutation carriers of this family ranged from classical asymmetric LV hypertrophy with or without clinical symptoms to three cases of progressive

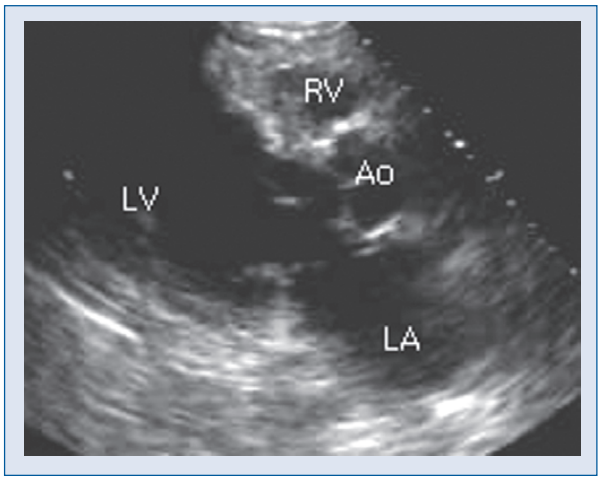

Figure 3. In 2002, one year prior to heart transplantation, severe dilatation with a left ventricular end-diastolic diameter of 65 mm, a further decrease of the ejection fraction to 30%, and a diminished width of the septum of 12 mm was described. The right ventricle seems to be smaller in size with slightly increased wall diameter compared to 1997, but this can be partially attributed to the cardiac cycle the frame was recorded in (mid-diastole). At that time, the pressure in the pulmonary circulation was moderately elevated; for abbreviations, see Figure 1.

LV dilation and end-stage systolic heart failure, which was in some cases age-dependent.

For molecular analysis, four tissue samples were taken from the right ventricle, and five biopsies from the anterior, lateral, and inferior wall and the septum of the left ventricle. We found a significant deviation from the expected 1:1 ratio between mutant and wild type myosin. This was similar for LV and right ventricular tissue (RV), with 71.8 ± 5% and 68.5 ± 3% respectively. The clinical phenotype on ECHO one year prior to transplantation, though, showed an asymmetric septal hypertrophy of the left ventricle and a systolic anterior movement of the anterior mitral valve (SAM). In addition, a pressure gradient of the LV outflow tract was present on provocation of 40 mm Hg. The dimensions and function of the right ventricle were described as normal. During follow up, in conformity to the progressive decline in the physical well-being of the patient, severe LV dilatation and systolic dysfunction was obvious on ECHO. But even at end stage LV failure, the right ventricle was not dilated and showed a normal function (Fig. 3).

Discussion

For several mutations of the β-MHC, current evidence indicates that there is an association between disease severity and the fraction of mutated β-myosin incorporated into the sarcomere [4]. We have previously shown that this fraction is specific for a given mutation on the mRNA and protein level [5, 6]. From this Catalan family (including patient II-5), Tripathi has analyzed the proportion of mutated β-MHC mRNA in LV tissue and *soleus* biopsies of three patients (two brothers and a nephew) and found a similar ratio of mutated *versus* wild type mRNA of 66% on average [4]. All three patients exhibited the phenotype characteristic of hypertrophic cardiomyopathy, but no overt RV disease. Thus, the relative amount of mutated β-MHC does not explain the discrepancy between the disease manifestation between the left and right ventricle.

The functional consequence of the Arg723Gly mutation on a cellular level was recently analyzed by force measurements on skinned *soleus* muscle fibers [7, 8]. The mutation results in reduced compliance and enhanced cross-bridge stiffness of the muscle fiber that is related to alterations in the most compliant region of the myosin head, the converter domain of the β-MHC. Several studies have confirmed that mutations in the β-MHC gene impair contractility and induce the release of growth factors that result in compensatory hypertrophy and fibroblast proliferation, the histologic hallmark of FHC [9]. Our findings show a similar expression of the mutant and wild type allele of the β-MHC in the left and right ventricle, but hypertrophy only of the left ventricle indicates that additional triggers are required for the initiation of the maladaptive compensatory response to the myocyte dysfunction. Such triggers could be environmental factors such as increased myocardial wall stress, which may explain why pathologic cardiac remodelling is predominantly restricted to the left ventricle as the chamber of maximal force generation and energy consumption [10]. Recently, research on the still unknown unifying mechanism for the development of cardiac hypertrophy in FHC has focused on the hypothesis that an imbalance of energy cost and supply resulting in a chronic 'energy compromise' state acts as a potent stimulus for hypertrophy [11]. The clinical observation that excess intraventricular pressure and thus enhanced energy demand of the ventricle is triggering myocardial hypertrophy was supported by the results of the two-year follow-up of FHC patients after elimination of their outflow tract gradient by septal myectomy, which induced a significant reduction in wall thickness, cardiac mass and myocardial collagen [12].

However, one plausible explanation for the difference in the abundance of myocardial hypertro-

phy in FHC between the left and right ventricles could also be the lack of adequate imaging tools, since the spatial resolution of two-dimensional echocardiography is limited and related to that, no definite standardized values for analysing RV dimensions and function have been published [13]. It was recently demonstrated in an imaging study using magnetic resonance imaging that morphologic abnormalities were present in 19 of 46 (41%) patients with FHC by showing diffuse RV wall thickening but no contractile dysfunction. And in only one of the 46 FHC patients included in the study, myocardial fibrosis as the typical hallmark of hypertrophic cardiomyopathy was detected by contrast magnetic resonance imaging as late enhancement areas in the right ventricle, whereas 26 of 46 patients (57%) had late enchancement areas within the LV myocardium.

The cardiomyopathy that predominantly involves the right ventricle is arrhythmogenic right ventricular cardiomyopathy (ARVCM), characterized by fibroadipocytic replacement of RV cardiac myocytes. Lombardi et al. [14] showed in a mouse model that second heart field progenitor cells act as the cell source of adipocytes in ARVCM. The right ventricle primarily originates from the second (anterior) heart field, as opposed to the left ventricle, which originates from the primary heart field. The two heart fields express distinct sets of transcriptional factors and signalling molecules [15]. It can be therefore speculated that the different developmental origins of the left and right ventricle play a role in the contrast disease manifestations between the two chambers. However, our data shows that the relative level of mutated β-MHC mRNA is not affected by the origin of the ventricles.

Future studies that integrate molecular, biological, functional and biochemical data from both the left and right ventricle should further elucidate the pathways by which the altered β-MHC results in pathologic cardiac remodelling. The mechanisms for the difference in disease manifestation, despite similar expression of the β-MHC mutation, should ultimately be translated into early therapeutic interventions to prevent the development of cardiac hypertrophy in mutation carriers.

Conclusions

Despite heterozygocity of the missense mutation Arg723Gly of the β-MHC, which causes severe hypertrophic cardiomyopathy, the ratio of mutant *versus* wild type myosin mRNA deviates from 1:1. This deviation is similar in both ventricles. Because of the evidence of a correlation between the malignancy of FHC and the fraction of mutant myosin expressed in the contractile apparatus, the reason for the development of myocyte hypertrophy primarily in the left ventricle in contrast to the right ventricle is unclear. Our data indicates that the induction of the process of pathologic cardiac remodeling and heart failure in FHC depends on additional influences, like environmental, humoral or genetic factors.

Acknowledgements

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