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Molecular and functional characterization of a human frataxin mutation found in hypertrophic cardiomyopathy

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Abstract

Hypertrophic cardiomyopathy is associated with marked genetic and phenotypic heterogeneity. Pathogenic mutations in the 10 hypertrophic cardiomyopathy-associated sarcomeric genes cause autosomal dominant disease as a rule, although recessive disease has been reported. Cardiac hypertrophy is also a hallmark of Friedreich ataxia, an autosomal recessive disease caused by deficiency of the mitochondrial protein frataxin. We hypothesized that heterozygous mutations in frataxin may mimic or modify hypertrophic cardiomyopathy. Using DHPLC and DNA sequencing, we identified the novel R40C-frataxin mutation in a patient who also harbored a previously reported R810H-myosin binding protein C mutation. The R810H mutation is reported to cause hypertrophic cardiomyopathy only in the setting of homozygosity or compound heterozygosity with another sarcomeric mutation. Site-directed mutagenesis and in vitro and in vivo analysis enabled functional characterization of the mutant frataxin protein. R40C-frataxin protein is not cleaved to the mature form in vitro and shows delayed kinetics of cleavage by isolated mouse mitochondria. Yeast cells expressing R40C-frataxin demonstrated increased sensitivity to oxidative stress and abnormal accumulation of precursor frataxin protein. These data indicate that frataxin deficiency may have contributed to this patient's particular phenotype. Furthermore, these findings suggest that mutations altering myocyte energetics may act in synergy with sarcomeric mutations to cause hypertrophic cardiomyopathy.

Keywords: Hypertrophic cardiomyopathy; Genetics; Myosin binding protein C; Frataxin; Friedreich ataxia

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Introduction

Hypertrophic cardiomyopathy (HCM) is a common disease diagnosed in 1:500 previously healthy young adults [1,2]. Our understanding of the pathogenesis of this disease is hampered by profound genetic heterogeneity and clinical manifestations ranging from lack of symptoms to sudden cardiac death at a young age [1,2]. Although called "a disease of the sarcomere," pathogenic mutations in sarcomeric genes have been identified in less than 40% of 389 patients with unequivocal and unexplained cardiac hypertrophy diagnosed at our institution [3]. When documented, inheritance is usually autosomal dominant, although recessive cases have been described [4,5]. Finally, a diverse spectrum of clinical presentations can be observed within families, indicating

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that unspecified disease modifiers may play a role in the pathogenesis of HCM [6].

Cardiac hypertrophy is frequently one manifestation of mitochondrial diseases [7], including Friedreich ataxia, an autosomal recessive neurologic and cardiac disease caused by deficiency of the mitochondrial protein frataxin [8]. In over 95% of Friedreich ataxia patients the molecular genetic defect involves homozygous trinucleotide repeat expansions in the first intron of the frataxin gene (*FRDA*), which impairs transcription and leads to <30% of normal frataxin levels. Patients with Friedreich ataxia usually develop symptoms around puberty with neurological symptoms almost always preceding cardiac involvement. Heart failure is the leading cause of death [9]. In yeast and human cells, frataxin deficiency leads to respiratory defects and increased sensitivity to oxidative stress [10]. These findings correlate with increasing evidence that frataxin plays a central role in mitochondrial iron metabolism and detoxification [11–13].

We hypothesized that heterozygous mutations in *FRDA* either mimic or modify the disease course of sarcomeric HCM. To test this hypothesis, we searched for mutations in *FRDA* in 389 patients with clinical HCM who were previously assessed for mutations in eight HCM-associated genes. The R40C-frataxin mutation was identified in one patient with HCM and a previously known myosin binding protein C mutation, R810H. In vitro characterization of R40C-frataxin revealed impaired processing to the mature form, and in vivo expression in yeast caused increased sensitivity to oxidative stress.

Methods

Patient cohort

The patient cohort studied has been described previously [14], and was comprised of 389 unrelated patients with unequivocal and unexplained cardiac hypertrophy (i.e., HCM). All protocols were approved by the Institutional Review Board, included written informed consent, and were in conformity with the Declaration of Helsinki. Comprehensive mutational analysis was completed for all protein-coding exons of the following genes: β-myosin heavy chain (*MYH7*), myosin binding protein C (*MYBPC3*), regulatory and essential light chains (*MYL2* and *MYL3*), troponin-T (*TNNT2*), troponin-I (*TNNI3*), α-tropomyosin (*TPM1*), and α-actin (*ACTC*) [3,14,15]. In this cohort, there were 65 patients with *MYBPC3* mutations, 55 *MYH7*, 7 *MYL2*, 6 *TNNT2*, 4 *TNNI3*, 2 *TPM1*, and 1 *ACTC*, as well as seven patients with mutations in >1 sarcomeric gene. No putative pathogenic mutations involving these eight sarcomeric genes were identified in the remaining 242 patients.

Mutation analysis

Each protein-coding exon of *FRDA* (GenBank Accession Nos. AI951739, BC023633, and BC048097) was amplified by PCR as previously described [16], and analyzed for mutations using denaturing high performance liquid chromatography (DHPLC) and direct DNA sequencing [17]. Amplification and direct sequencing from stock vials of patient DNA confirmed all sequence variants. DNA from 100 apparently healthy whites and 100 apparently healthy blacks (Coriell Institute, Camden, NJ) provided 400 reference alleles to exclude candidate *FRDA* mutation(s) as a common nonsynonymous polymorphism.

Clinical examination

A multidisciplinary team at our institution examined the patient with the R40C-frataxin mutation. The *FRDA* trinucleotide repeat expansion size was normal on both alleles.

In vitro processing assays

A pGEM-3Z vector containing the wild-type or R40C-frataxin cDNA was generated by PCR-mediated site-directed mutagenesis. Primer sequences and cycling conditions are available upon request. Direct DNA sequencing confirmed that correct DNA sequence was present throughout the *FRDA* cDNA. In vitro transcription and translation was performed using the TNT Coupled Reticulocyte Lysate System (Promega, Madison, WI) per manufacturer's protocol. SDS–PAGE and scintillation counting analyses were employed to confirm equivalent amounts of radiolabeled protein were present in the wild-type and R40C-frataxin samples (not shown). Purified yeast mitochondrial processing peptidase (MPP) was generated and enzyme activity assayed using methods described previously in detail [18]. Protein import assays were performed with freshly isolated mouse liver mitochondria [18].

Characterization of R40C-Frataxin yeast

Previously, a YCplac22 vector carrying the wild-type *FRDA* cDNA with the yeast glyceraldehyde-3-phosphate dehydrogenase promoter and *TRP1* selectable marker was generated and designated YC-FRDA [19]. Using PCR-mediated site-directed mutagenesis, the R40C-frataxin point mutation was introduced to create YC-FRDA[R40C]. Both constructs were confirmed by DNA sequencing. Yeast strains expressing the wild-type and R40C-frataxin precursor protein were generated as previously described [19] and designated *yfh1*Δ[FRDA] and *yfh1*Δ[FRDA-R40C], respectively. Phenotypic differences between *yfh1*Δ[FRDA] and *yfh1*Δ[FRDA-