Isolated X-Linked Hypertrophic Cardiomyopathy Caused by a Novel Mutation of the Four-and-a-Half LIM Domain 1 Gene

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Background—Hypertrophic cardiomyopathy with severe left ventricular diastolic dysfunction has been associated with marked exercise intolerance and poor prognosis. However, molecular pathogenesis of this phenotype remains unexplained in a large proportion of cases.

Methods and Results—We performed whole exome sequencing as an initial genetic test in a large Czech family with 3 males affected by nonobstructive hypertrophic cardiomyopathy with severe left ventricular diastolic dysfunction in end-stage disease. A novel frameshift mutation of four-and-a-half LIM domain 1 gene (FHL1) (c.599_600insT; p.F200fs32X) was detected in these individuals. The mutation does not affect transcription, splicing, and stability of FHL1 mRNA and results in production of truncated FHL1 protein, which is contrary to heart tissue homogenate not detectable in frozen tissue sections of myocardial biopsy of affected males. The identified mutation cosegregated also with abnormal ECG and with 1 case of apical hypertrophic cardiomyopathy in heterozygous females. Although skeletal muscle involvement is a common finding in FHL1-related diseases, we could exclude myopathy in all mutation carriers.

Conclusions—We identified a novel FHL1 mutation causing isolated hypertrophic cardiomyopathy with X-chromosomal inheritance.

Key Words: cardiomyopathy, hypertrophic \# exome \# heart failure, diastolic

Severe left ventricular diastolic dysfunction (LVDD) in individuals with hypertrophic cardiomyopathy (HCM) has been associated with poor exercise tolerance and prognosis in both adults and children with this disease.\textsuperscript{1–5} Severe LVDD may occur as an initial manifestation of HCM with limited LV hypertrophy, or it may represent advanced disease, an alternative to end-stage dilated HCM.\textsuperscript{6} Recently, mutations of β-myosin heavy chain, cardiac troponin I,\textsuperscript{7,8} and cardiac troponin T\textsuperscript{9} have been identified as disease genes for HCM with severe LVDD.

Original Article

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We report on a large family with isolated X-linked HCM with severe LVDD cosegregating with a novel mutation of FHL1 gene encoding four-and-a-half LIM domain protein 1 (FHL1). More than 30 mutations of FHL1 have been associated with 5 different X-linked myopathies—reducing body myopathy (MIM 300718), scapuloperoneal myopathy (MIM 300695), X-linked myopathy with postural muscle atrophy (MIM 300696), rigid spine syndrome, and Emery-Dreifuss muscular dystrophy (MIM 310300) with a variable cardiac involvement presenting as dilated or HCM.\textsuperscript{10–14} In addition, a recent report described 2 novel variants of FHL1 causing isolated HCM.\textsuperscript{15} This report extends the spectrum of FHL1-related diseases by description of FHL1 as a causal gene for isolated HCM with severe LVDD in advanced disease.

Methods

Study Population
The index patient was identified in a large Czech family with 3 males affected by HCM who developed severe LVDD and advanced heart failure in 2 cases. A detailed family history was obtained, and an extended pedigree was constructed (Figure 1) according to the guidelines.\textsuperscript{16} Further investigations were approved by the Institutional Review Boards of the participating center and were performed according to the Declaration of Helsinki principles. Written informed consent was obtained from all subjects.

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Cardiological Screening
We examined 20 members of the above family. Medical records were acquired in the proband’s uncle who was treated in our institution and died after heart transplantation. The cardiological screening included physical examination, electrocardiography, and echocardiography. Echocardiography was performed by a single operator (T.M.) in accordance with guidelines of the American Society of Echocardiography.17,18 M-mode, 2-dimensional images, and conventional and tissue Doppler recordings were obtained using a Vivid 7 (GE Healthcare, Chalfont St Giles, United Kingdom), LV ejection fraction was assessed using Simpson biplane method. Mitral inflow pattern was classified as restrictive in the presence of E-wave deceleration time <120 ms or a ratio of early transmitral flow velocity to atrial flow velocity ≥ 1.5.65.

The proband and his brother underwent cardiovascular magnetic resonance (Siemens Trio scanner; Siemens Medical Solutions, Erlangen, Germany). Ventricular volumes, ventricular mass, and ejection fractions were obtained from standard cine images in short-axis (repetition time/echo time, 65/1.2 ms; slice thickness of 8 mm without interslice gap) using a dedicated software Segment 1.8 (http://segment.edu/EVS/), and internal exome database were prioritized for further analysis. Identified genetic variants were filtered according to an autosomal dominant or X-linked genetic model of the disease, which was inferred from the pedigree structure, and the resulting 26 candidate coding variants were evaluated in GeneDistiller,24 according to biological relevance of corresponding genes to cardiomyopathy. Candidate variants were visualized in Integrative Genomics Viewer, version 1.5.65. Mutation-bearing fragment of FHL1 (NM_001159702) was polymerase chain reaction (PCR) amplified from genomic DNA of all available individuals from the family and sequenced using version 3.1 Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) with electrophoresis on an ABI 3500XL Avant Genetic Analyzer (Applied Biosystems). Data were analyzed using Sequencing Analysis software, and the segregation of the candidate FHL1 mutation with the phenotype was assessed.

Analysis of Myocardial Samples

Material Collection
Myocardial specimens from hearts explanted during transplantation were available in the proband (II/4) and his uncle (II/3). Samples from all major anatomic locations of the proband's heart were prospectively saved for histopathology, immunohistochemistry, and electron microscopy and were also snap-frozen for molecular analyses. Explanted heart from the male patient II/3 was available as formaldehyde-fixed paraffin-embedded tissue from all major anatomic heart locations and small snap-frozen samples for molecular analyses from an another research project.

Control Samples
Snap-frozen myocardial specimens were obtained from 4 hearts explanted during heart transplantation. Underlying cardiac disease was dilated cardiomyopathy in control 1, an 18-year-old female, and control 2, a 52-year-old male. Ischemic heart disease had control 3, a 53-year-old male, and control 4, a 52-year-old female.

Histology and Immunohistochemistry
Formaldehyde-fixed paraffin-embedded tissue samples were sectioned (4 µm) and processed for histology and immunohistochemistry. The paraffin sections were stained by standard histological techniques (hematoxylin-eosin, trichrom stain). Immunohistochemistry of sarcomeric actin, desmin, lysosomal-associated membrane proteins 1 and 2, and β subunit of mitochondrial ATP synthase (ATP B) was applied on selected paraffin sections. Immunohistochemical findings were evaluated in comparison with those seen in a set of previously examined myocardial specimens diagnosed for cardiomyopathies based on mitochondrial disorders, lysosomal storage disorders, and amyloidosis, and for dilated or HCM not otherwise specified for a genetic defect.

Immunohistochemical Analysis of FHL1 Protein
The monoclonal antibody detecting FHL1 (Ab58067; Abcam, Cambridge, UK) was tested in both paraffin and cryostat sections of myocardium and skeletal muscle, including demasking of antigen epitopes in formaldehyde-fixed paraffin-embedded tissue material. Specific and sensitive results were obtained only in unfixed frozen

Molecular Genetic Analysis
Genomic DNA of all available individuals was extracted from whole blood samples using a standard technology. Genomic DNA of the proband’s uncle was available from a previous research project.21 With respect to known genetic heterogeneity of familiar cardiomyopathies, the whole exome sequencing was performed as an initial genetic test. Exome sequencing was performed using 2 µg of DNA from 3 affected individuals (II/3, II/4, and II/5). For DNA enrichment, individually bar-coded DNA libraries22 and SureSelect All Exome Kit V4 (Agilent Technologies, Santa Clara, CA) were used according to the manufacturer protocol. DNA sequencing was performed on the captured bar-coded DNA library using SOLID 4 System (Applied Biosystems, Carlsbad, CA) at the Institute for Inherited Metabolic Disorders (Prague, Czech Republic). Fifty base pair reads were aligned in color space to the reference genome (hg19) using NovoalignCS version 1.08 (Novocraft, Petaling Jaya, Malaysia) allowing for ≤6 mismatches. Sequence variants in analyzed samples were identified using SAMtools package (version 0.1.8).24 The high confidence variants (quality ≥50 and coverage ≥10x) were annotated using ANNOVAR Annotation tool (hg19). Only the sequence variants present in all 3 affected individuals and having frequency ≤0.001 in the single-nucleotide polymorphism database, 1000 Genomes, Exome Variant Server (http://evs.gs.washington.edu/EVS/), and internal exome database were prioritized for further

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tissues. Immunohistochemical detection of FHL1 was therefore performed in cryostat sections of myocardium from the proband III/4. Frozen samples from male patient II/3 were no longer available for immunohistochemical analysis. Cryostat sections (5 μm) fixed in cold anhydrous acetone for 10 minutes at −20°C were dried for 20 minutes at room temperature, then underwent standard blocking procedures. Primary mouse monoclonal antibody (Ab58067; Abcam) was applied diluted 1:100 in 5% BSA in PBS. Sections were incubated for 1 hour at 37°C with the primary antibody and then washed in PBS. Detection of bound primary antibody was achieved using Dako EnVision+System-HRP Mouse (Dako, Glostrup, Denmark) with 3,3-diaminobenzidine as a substrate.

**Electron Microscopy**

Samples fixed in 10% paraformaldehyde were subsequently embedded into an Epon-Araldite mixture, double stained, and examined using a Jeol 1200 electron microscope.

**Qualitative (Reverse Transcriptase PCR) Analysis of FHL1 mRNA**

Total RNA was isolated from snap-frozen myocardial specimens using the TRIZOL solution (Invitrogen, Carlsbad, CA). RNA concentration was determined spectrophotometrically at 260 nm by NanoDrop (NanoDrop Technologies, Wilmington, DE), and quality was checked on Agilent 2100 bioanalyzer—RNA Laboratory-On-a-Chip (Agilent Technologies). Aliquots of isolated RNA were stored at −80°C until analysis. The first-strand cDNA synthesis was performed using oligo-dT primer and SuperScript III Reverse Transcriptase (Life Technologies, Carlsbad, CA). FHL1 cDNA was PCR-amplified from the synthesized first-strand cDNA using oligonucleotide primers cFHL1_1135 5’-CCCATCCTGTTGCGACTCCAAG-3’ and cFHL1_1013L 5’-TTTGCCACAGTCGGACAATA-3’ designed to span and amplify all 3 FHL1 mRNA isoforms in parallel. Obtained PCR products were sequenced using version 3.1 Dye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) with electrophoresis on an ABI 3500XL Avant Genetic Analyzer (Applied Biosystems).

**Quantitative PCR Analysis of FHL1 mRNA**

The reactions were performed in a 96-well plate in a 20-μL reaction volume containing 10 μL of 2×Maxima SYBR Green quantitative PCR Master Mix (Thermo Scientific); 0.2 μmol/L of cFHL1_135U and cFHL1_1013L primers and 5 ng of cDNA on a StepOne Plus Real Time System (Applied Biosystems). Data were analyzed by StepOne Software v 2.0. The comparative Ct (ΔΔ Ct) method was used to normalize FHL1 mRNA to GAPDH mRNA amounts.

**Western Blot Analysis of FHL1 Protein**

Frozen myocardial specimens were homogenized under liquid nitrogen, dissolved in 10 mmol/L Tris, 10 mmol/L KCl, 2 mmol/L EDTA, 4% glycerol, 1 mmol/L DTT, Complete Protease Inhibitor Cocktail (Roche Diagnostics, Basel, Switzerland), centrifuged at 15 000 g for 30 minutes at 4°C, and assessed for protein content in the supernatant using the Bradford assay. Homogenate aliquots corresponding to 30 μg of total protein were resolved on 10% SDS-PAGE and transferred using the Bradford assay. Homogenate aliquots corresponding to 30 μg of total protein were resolved on 10% SDS-PAGE and transferred using the Bradford assay. Sections were incubated for 1 hour at 37°C with the primary antibody and then washed in PBS. Detection of bound primary antibody was achieved using Dako EnVision+System-HRP Mouse (Dako, Glostrup, Denmark) with 3,3-diaminobenzidine as a substrate.

**Neurological Assessment**

All living individuals with FHL1 mutation underwent neurological assessment focused on detection of possible muscle disability (muscle atrophy, muscle strength testing), including standard routine examination of the central and peripheral nervous systems. In adult patients with FHL1 mutation, nerve conduction studies of the motor and sensory nerves of lower extremities were performed to exclude neuropathy. Two muscles (left vastus medialis and left deltoid muscle) were investigated by needle electromyography. Electromyographic signals were evaluated using commercially available analyzer Medelec Synergy (Viasis Neurocare, Madison, WI) to detect possible muscle disease. In addition, all living individuals with FHL1 mutation had a biochemical analysis to assess creatine phosphokinase activity and myoglobin concentration in serum.

**Results**

**Clinical Features of the Proband**

The proband was a 31-year-old male (III/4) with a history of nonobstructive HCM since 18 years of age who presented with dyspnea in New York Heart Association functional class IV, right heart failure, and new-onset atrial fibrillation with a rapid ventricular response. Symptoms of end-stage heart failure persisted even after successful cardioversion to sinus rhythm. Proband’s ECG showed signs of biatrial enlargement and LV hypertrophy (Figure 2). In comparison with previous data (Table), the most recent echocardiography study demonstrated nonobstructive HCM with less prominent LV hypertrophy, preserved LV systolic function, and severe LVDD. Cardiac catheterization revealed normal coronary arteries, low cardiac output, elevated filling pressures of both ventricles, and borderline pulmonary artery pressures (Table). These findings were compatible with advanced heart failure attributable to nonobstructive HCM with severe LVDD. The patient underwent an uneventful heart transplantation 6 months later. His family history revealed diagnosis of HCM in his brother and uncle.

**Pedigree Analysis**

**Male Patient III/5**

Brother of the proband was diagnosed with nonobstructive HCM at 17 years of age. At 30 years of age, he reported mild breathlessness. ECG showed first degree of atrioventricular block, signs of biatrial enlargement, and LV hypertrophy (Figure 2). Echocardiographic findings were compatible with nonobstructive HCM with severe LVDD (Table).

**Male Patient II/3**

Uncle of the proband was hospitalized due to decompensated heart failure at 55 years of age. Subsequent cardiological examination revealed nonobstructive HCM with severe LVDD disproportional to mild-to-moderate LV systolic dysfunction (ejection fraction 40%–45%; Table; Figure 3). At 59 years of age, he was admitted to our institution because of end-stage...
heart failure and underwent an urgent heart transplantation. Unfortunately, the postoperative course was complicated by severe right ventricular failure, hepatorenal failure, and sepsis. The patient died 4 months after transplantation.

**Female Patient I/2**

Grandmother of the proband was diagnosed with an apical form of HCM during cardiological screening in the family. This 83-year-old lady reported a history of myocardial infarction, diabetes mellitus, and breathlessness New York Heart Association class III; however, she did not experience any episode of heart failure. Echocardiography revealed apical hypertrophy of the left ventricle beginning in the midventricular segment (septum, 14 mm; lateral wall, 12 mm) reaching 15 to 16 mm in the apical third (Figure 3). The patient refused MRI and any other examinations.
Results of the Cardiological Screening

Cardiological examination in the remaining family members revealed electrocardiographic signs of LV hypertrophy (Figure 2) in 2 asymptomatic females heterozygous for FHL1 mutation (II/1: aunt of the proband, 63 years of age; II/2: mother of the proband, 53 years of age). Echocardiography demonstrated normal findings in both females except for modest increase in LV mass (98 and 90 g/m², respectively). Mild electrocardiographic abnormalities, such as mild depression of ST segments and either negative or biphasic T waves in leads III and aVF, were detected in 2 other females heterozygous for FHL1 mutation (III/1: 41 years of age; III/8: 34 years of age) and 1 male hemizygous for FHL1 mutation (IV/2: 17 years of age). All these subjects were asymptomatic and had normal echocardiographic findings. The results of the cardiological screening were normal in the remaining members of the family, including a female heterozygous for FHL1 mutation (IV/1: 20 years of age) and a male heterozygous for FHL1 mutation (IV/9: 2 years of age).

Genetic and Molecular Biology Analyses

To directly identify possible disease-causing mutation, we performed exome sequencing in 3 affected individuals (II/3, III/4, and III/5). From the sequencing run, we obtained from these samples 128, 151, and 109M of sequencing reads, respectively, of which on average 57% we were able to map uniquely on the human genome reference sequence (hg19). Compared with the reference sequence, we identified in these data 16486, 17953, and 16591 variants, respectively. Among them, we identified 8515 single-nucleotide variants (SNVs; single-nucleotide polymorphism/indel qual >50) that were present in all 3 affected probands. From these variants, 69 SNVs (2 stop-gain, 46 nonsynonymous SNVs, and 21 synonymous SNVs) and 3 indels were either novel or present at frequencies <0.01 in the 1000 Genomes, Exome Variant Server (http://evs.gs.washington.edu/EVS/), and internal exome database (>200 exomes). Resulting 51 rare coding variants, 48 SNVs, and 3 indels (Table I in the online-only Data Supplement) were further evaluated in GeneDistiller (24) according to potential biological and clinical relevance of corresponding genes to cardiomyopathy. From this evaluation emerged the only potentially causative variant, the frameshift insertion c.599_600insT in exon 6 of FHL1 (NCBI reference sequence: NM_001159702). FHL1 encodes the four-and-a-half LIM domain protein 1 (FHL1). The gene is transcribed into 3 alternatively spliced mRNA isoforms FHL1A, FHL1B, and FHL1C, encoding FHL1A, FHL1B, and FHL1C proteins, respectively. FHL1A is by far the most abundant form. It is highly expressed in skeletal muscle, moderately in heart, and to a much lesser extent in a wide array of other tissues.10,11 Compared with FHL1A, the other 2 isoforms are expressed at much lower abundance and demonstrate tissue-specific expression patterns. FHL1B is specifically expressed in the brain, whereas FHL1C is expressed in skeletal muscle and at lower levels in the aorta, left atrium, and left and right ventricles of the human heart.11,25 The insertion encodes for a frameshift in translation of FHL1A and FHL1B isoforms, which are in both cases followed soon thereafter by a novel stop codon predicting proteosynthesis of 1 sequentially identical truncated form of the FHL1A containing a neopeptide composed of 32 aminoacids (200 HRCGGPVLLRGLLQEL-CGQEV-CWMQEPHVVWV-232) on the C-terminal end (p.F200fs32X). Located in alternatively spliced exon 6, the identified insertion is not predicted to affect proteosynthesis of the FHL1C variant (Figure 4A). We confirmed the presence of the c.599_600insT variant of FHL1 in genomic DNA of probands by Sanger sequencing (Figure 4B), and using the same technique, we subsequently demonstrated segregation of the mutation with the phenotype of cardiac disease in the family (Figure 1).

To characterize molecular consequences of the identified mutation, we assessed expression of FHL1 mRNA isoforms and presence of FHL1 proteins in patients and control tissues obtained from 2 females (C1F and C4F) and 2 males (C2M and C3M). We performed reverse transcription PCR and quantitative PCR analysis of total RNA extracts and Western blot analysis of protein homogenates prepared from the snap-frozen myocardial specimens. Quantitative PCR analysis suggested that FHL1 transcription may be generally higher in females than in males and that the mutation and related dysfunction of FHL1 protein may stimulate FHL1 transcription in affected males (Figure 4C). Reverse transcription PCR analysis revealed in all analyzed myocardial specimens the presence of a single reverse transcription PCR product of the size expected for FHL1A isoform (Figure 4D). The Sanger sequencing and sequence analysis demonstrated that the obtained reverse transcription PCR products correspond to FHL1A mRNA isoform and independently confirmed in patients’ samples the presence of the frameshift insertion c.599_600insT identified previously in corresponding genomic DNA (not shown). In accordance with this finding, Western blot analysis of myocardial homogenates revealed presence of the immune-reactive protein of a molecular weight ≈27 kDa corresponding to predicted molecular weight of the p.F200fs32X FHL1 protein in patients’ samples. Immunoreactive protein of a molecular weight ≈32 kDa,
corresponding to predicted molecular weight of the FHL1A identified in control samples, was absent in patients’ samples. Immunoreactive protein of a molecular weight ≈22 kDa, corresponding to predicted molecular weight of the FHL1C, was not detected. Instead, the analysis revealed presence of the immunoreactive protein of a molecular weight ≈25 kDa in all controls’ and patient’s samples, which identity is unknown (Figure 4E). In contrast to Western blot, the immunohistochemical staining of cryostat sections of myocardium revealed absence of any immunoreactive FHL1 protein (Figure 4F) in the proband III/4 in comparison with distinct cross-striation pattern observed in control myocardium (Figure 4G).
Morphological Findings in 2 Explanted Hearts

**Male Patient III/4 (Proband)**
The explanted heart weighed 346 g (less than the upper limit of normal 400 g). On gross pathology, both ventricles were nondilated with relatively thin walls (left ventricle, 9–12 mm; right ventricle, 5 mm) and discrete focuses of fibrosis on incision. Both atria were dilated, with limited amounts of tissue missing attributable to surgical reasons. Coronary vessels showed mild intimal thickening. Histopathology revealed hypertrophy of cardiomyocytes with anisonucleosis and hyperchromasia, interstitial and replacing fibrosis, subendocardial scars and vacuolization of cardiocytes, and mild sclerosing arteriosclerosis. Disarray of cardiomyocytes was present, involving 5% to 15% of the tissue section in the LV wall (Figure 5A, 5C, and 5E). Sarcomeric actin and desmin detected immunohistochemically revealed an usual cross-striation pattern without detectable inclusions, being slightly disordered in focuses of myocyte disarray (Figure 5F). There were no abnormalities of lysosomal and mitochondrial compartments based on immunohistochemistry. Electron microscopy showed prevalent regular organization of myofibrils and increased number of mitochondria in subsarcolemmal localization or focally replacing myofibrils. Mitochondria did not show any substantial abnormalities in size, shape, or organization of their crists. Disorganized myofibrils in cardiocytes were found exceptionally (Figure 5G and 5H). Morphological findings were comparable in both ventricles.

**Male Patient II/3 (Proband’s Uncle)**
The explanted heart weighed 578 g (the upper limit of normal 400 g). Gross pathology showed mild dilatation of both ventricles with thickened LV wall (10–18 mm), dilated atria, and mild atherosclerotic plaques of coronary arteries. Histopathology demonstrated hypertrophy of cardiomyocytes, focal myocyte disarray less frequent than in the proband, and marked interstitial and replacing fibrosis (Figure 5B and 5D). Immunohistochemical staining for sarcomeric actin and desmin showed patterns comparable with that in the proband. No abnormalities of lysosomal and mitochondrial systems were detected using immunohistochemistry. The histopathologic changes involved both ventricles.

In summary, both patients, the proband (III/4) and his uncle (II/3), showed a different degree of hypertrophy and disarray of cardiocytes together with interstitial fibrosis, which are features consistent with HCM at the histological level.

Neurological Assessment
Mutations in FHL1 have been identified as the cause of several skeletal muscle diseases. In this respect, carriers of the c.599_600insT mutation underwent neurological examination. Neurological status of individuals with FHL1 mutation was unremarkable, except for mild muscle hypotrophy in the proband. However, needle electromyographic recordings as well as nerve conduction studies showed normal findings. Similarly, biochemical markers of muscle injury in peripheral blood had normal values in all individuals with FHL1 mutation. Taken together, there were no signs of muscle disease in these individuals.

Discussion
In this report, we describe a Czech family in which frameshift mutation c.599_600insT in exon 6 of FHL1, a gene located on chromosome X, cosegregated with nonobstructive HCM with severe LVDD in advanced disease in 3 hemizygous males. Penetration of this mutation was age dependent and included manifestation of HCM in hemizygous males in the second to sixth life decade and progression into advanced heart failure in 2 of these individuals in the fourth to sixth decennium. On the contrary, mild cardiac involvement in heterozygous females, consistent with random X-inactivation of mutated FHL1, included asymptomatic abnormalities of ECG with a modest increase in LV mass since the sixth decennium and 1 case of apical HCM in the ninth decennium. Microscopic features compatible with HCM were detected in both cases available for histopathologic analyses. None of the individuals showed signs of skeletal muscle disease.
Molecular Consequences of c.599_600insT FHL1 Mutation

The above mutation is either rare or even private because it has not been observed in any of the publically searchable human genome, exome, or polymorphism and mutation databases as well as in our internal population-specific human exome sequence database (>200 exomes). The mutation does not affect synthesis and stability of FHL1A mRNA in heart tissue, and the transcript seems to be translated into truncated 27 kDa form of the FHL1 protein, which is probably missing LIM3 and LIM4 domains and containing the neopeptide composed of 32 aminoacids on C terminus. In parallel, the mutation that is predicted to abolish proteosynthesis of FHL1A and FHL1B proteins should not have an impact on proteosynthesis of FHL1C.

In our study, we have not found any evidence for expression of FHL1C mRNA isoform and production of FHL1C protein (of predicted molecular weight) in analyzed control and patient-derived myocardial specimens. Instead, we detected in all these samples the presence of an immunoreactive protein of a molecular weight ≈25 kDa. Origin of this protein is unknown, and it remains to be investigated whether it represents post-translationally modified FHL1C isoform, proteolytically processed FHL1 protein, or simply nonspecificity of the antibodies used in this study. The amounts of the p.F200fs32X FHL1 protein in homogenates of patients myocardial specimens were comparable with amounts of FHL1A in control samples. Contrary to this observation, we found reduction of FHL1 signal in frozen tissue sections from patient specimens. Decrease in FHL1 staining in this material thus suggests that p.F200fs32X mutation results in proteosynthesis of truncated neoprotein, which has, either because of loss of LIM3 and LIM4 domains or because of presence of the neopeptide on its C-terminal end, altered structural properties limiting function and immunodetection of the mutant protein in the heart tissue.

Reasons for Preserved Skeletal Muscle Function in p.F200fs32X FHL1 Mutation

Our finding of isolated HCM resulting from complete structural and functional deficiency of FHL1A demonstrates different biological and functional roles of FHL1A proteins in skeletal and heart muscles. In skeletal muscle, it has been shown that FHL1 may have multiple roles in myoblast migration and elongation, satellite cell activation, inhibition of myoblast apoptosis, regulation of skeletal muscle mass, sarcomere formation, and Notch signaling.10,11 Clinical studies revealed that C-terminal FHL1 mutations preserving synthesis of FHL1C are responsible for less severe myopathic phenotype than the N-terminal ones.10,26 Importantly, FHL1C isoform seems to be expressed much stronger in human skeletal muscle than in myocardium.25 Preserved expression of the FHL1C isoform in skeletal muscle may explain absence of myopathy in our case. Unfortunately, we had no opportunity to analyze skeletal muscle specimens in our patients.

In addition, missense mutations disrupting zinc-binding residues critical for tertiary structure of FHL1 result in more severe myopathy than FHL1 truncations.10 This can be illustrated by replacement of highly conserved cysteine binding a zinc-ion within LIM3 domain (c. 625T>C; p.C209R). Although this mutation affects the same exon and LIM domain as in our case, it causes not only HCM but also Emery-Dreifuss myopathy with cytoplasmatic bodies suggesting misfolding of mutated FHL1 protein.13 This observation provides an alternative hypothesis that mutations leading to reduced amounts (or even absence) of FHL1 proteins are less deleterious (or even benign, as in our case) for skeletal muscle than missense mutations exerting their pathogenic effects through misfolding, self-aggregation, and coaggregation of FHL1-binding partners.10,11,14

Practical Implications

HCM with severe LVDD has been associated with mutations of myofilament proteins (β-myosin heavy chain, cardiac troponin I, cardiac troponin T, α-cardiac actin)18 and Z-disk proteins (myopalladin).27 However, myofilament mutations explained ≈50% cases of HCM with severe LVDD in the abovementioned study.12 In our study, phenotype of HCM with severe LVDD seemed to be associated rather with advanced disease than to be a primary phenotype of the abovementioned FHL1 mutation. As shown by Friedrich et al,15 FHL1 mutations may explain pathogenesis of isolated HCM in 2.5% of cases unexplained by traditional sarcomeric mutations. In addition, a recent report of Binder et al28 extends the spectrum of FHL1-related diseases by description of spongiiform HCM in individuals with X-linked myopathy with postural muscle atrophy. In summary, mutation of FHL1 should be suspected in individuals with X-linked HCM. FHL1 mutations thus extend spectrum of X-linked HCM where belong Barth syndrome, Danon and Fabry disease. Exome sequencing seems to be the most practical approach in genetic dissection of familiar cardiomyopathies.

Study Limitations

Unfortunately, we did not have an opportunity to study the pathophysiology of p.F200fs32X FHL1 mutation in experimental models. However, perfect segregation of the mutation in 12 individuals, available evidence in other FHL1-related diseases, and absence of wild-type FHL1A protein in myocardial samples support involvement of the above mutation in the pathophysiology of HCM in our case. Finally, the disease severity may be determined not only by the gene mutation itself but also by gene dosage and age. Further studies are needed to evaluate the relationship between the above gene mutation and LVDD observed in our patients.

Conclusions

We identified a novel mutation of FHL1 (c.647_648 ins:T) causing isolated HCM with X-chromosomal inheritance and severe LVDD in advanced disease in 3 hemizygous males. Mild cardiac involvement in heterozygous females included asymptomatic abnormalities of ECG with a modest increase in LV mass since the sixth decennium and 1 case of apical HCM in the ninth decennium. None of the individuals showed signs of skeletal muscle disease. Mutations of FHL1 should be suspected in individuals with X-linked HCM.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

The study identified a novel molecular cause of isolated X-linked hypertrophic cardiomyopathy (HCM) caused by frame-shift mutation of four-and-a-half LIM domain 1 gene (FHL1). FHL1 gene mutations have been previously associated mainly with X-linked myopathies with variable cardiac involvement. However, rarely, they may also cause isolated HCM. The study extends spectrum of X-linked HCM where belong Barth syndrome, Danon, and Fabry disease. In addition, affected males with FHL1-related HCM presented with severe left ventricular diastolic dysfunction and advanced heart failure in end-stage disease. Consequently, FHL1 gene mutation should also be considered in cases of severe diastolic heart failure in end-stage HCM.
Isolated X-Linked Hypertrophic Cardiomyopathy Caused by a Novel Mutation of the Four-and-a-Half LIM Domain 1 Gene
Hana Hartmannova, Milos Kubanek, Marek Sramko, Lenka Pihrova, Lenka Noskova, Katerina Hodanova, Viktor Stranecky, Anna Pristoupilova, Jana Sovova, Tomas Marek, Jana Maluskova, Petr Ridzon, Josef Kautzner, Helena Hulkova and Stanislav Kmoch

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Supplemental Material
**Supplemental Table 1:** Novel and rare genetic variants identified by exome sequencing as present in all three analyzed probands.

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Individual variants are defined by chromosomal localization (Chr), their chromosomal position (Position), reference (Ref) and observed (Ob) alleles, gene symbol (Gene), accession number of the corresponding cDNA (Accession), resulting cDNA and amino acid changes, population frequency in exome sequence project (ESP5400) and predicted effect on protein function according to SIFT and Polyphen2 algorithms. In SIFT prediction D denotes
damaging effect and T denotes tolerating effect. In PolyPhen2 prediction B denotes benign effect, P denotes possibly damaging effect and D denotes probably damaging effect. All coordinates refer to hg19.