ORIGINAL ARTICLE

Glycogen Storage Diseases Presenting as Hypertrophic Cardiomyopathy

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ABSTRACT

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BACKGROUND

Unexplained left ventricular hypertrophy often prompts the diagnosis of hypertrophic cardiomyopathy, a sarcomere-protein gene disorder. Because mutations in the gene for AMP-activated protein kinase γ_2 (*PRKAG2*) cause an accumulation of cardiac glycogen and left ventricular hypertrophy that mimics hypertrophic cardiomyopathy, we hypothesized that hypertrophic cardiomyopathy might also be clinically misdiagnosed in patients with other mutations in genes regulating glycogen metabolism.

METHODS

Genetic analyses performed in 75 consecutive unrelated patients with hypertrophic cardiomyopathy detected 40 sarcomere-protein mutations. In the remaining 35 patients, *PRKAG2*, lysosome-associated membrane protein 2 (*LAMP2*), α-galactosidase (*GLA*), and acid α-1,4-glucosidase (*GAA*) genes were studied.

RESULTS

Gene defects causing Fabry's disease (*GLA*) and Pompe's disease (*GAA*) were not found, but two *LAMP2* and one *PRKAG2* mutations were identified in probands with prominent hypertrophy and electrophysiological abnormalities. These results prompted the study of two additional, independent series of patients. Genetic analyses of 20 subjects with massive hypertrophy (left ventricular wall thickness, ≥30 mm) but without electrophysiological abnormalities revealed mutations in neither *LAMP2* nor *PRKAG2*. Genetic analyses of 24 subjects with increased left ventricular wall thickness and electrocardiograms suggesting ventricular preexcitation revealed four *LAMP2* and seven *PRKAG2* mutations. Clinical features associated with defects in *LAMP2* included male sex, severe hypertrophy, early onset (at 8 to 17 years of age), ventricular preexcitation, and asymptomatic elevations of two serum proteins.

CONCLUSIONS

LAMP2 mutations typically cause multisystem glycogen-storage disease (Danon's disease) but can also present as a primary cardiomyopathy. The glycogen-storage cardiomyopathy produced by *LAMP2* or *PRKAG2* mutations resembles hypertrophic cardiomyopathy but is distinguished by electrophysiological abnormalities, particularly ventricular preexcitation.

HYPERTROPHIC CARDIOMYOPATHY, AN autosomal dominant disorder associated with increased morbidity and premature mortality, is traditionally diagnosed on the basis of increased cardiac mass with histopathological findings of myocyte enlargement, myocyte disarray, and cardiac fibrosis.[1-3] However, given the availability of sophisticated noninvasive imaging techniques, an echocardiographic demonstration of unexplained left ventricular hypertrophy constitutes the current basis for a diagnosis of hypertrophic cardiomyopathy.[3] Echocardiography has shown that there is considerable diversity in the manifestations of hypertrophic cardiomyopathy, including variable age at onset, from early childhood to late adulthood, and severity of left ventricular hypertrophy. Left ventricular wall thickness in hypertrophic cardiomyopathy can vary from slightly above normal to more than 50 mm (range, 13 to 60 mm), and massive hypertrophy (left ventricular wall thickness, ≥30 mm) is increasingly recognized as an important risk factor for sudden death.[3,4]

Sarcomere-protein gene mutations cause familial or sporadic hypertrophic cardiomyopathy and 15 percent of the cases of elderly-onset hypertrophic cardiomyopathy.[5] To date, more than 200 mutations in 10 different genes are known.[6] Molecular studies of patients with clinical features of hypertrophic cardiomyopathy but without sarcomere-protein gene defects have led to the identification of other genetic causes of cardiac hypertrophy, including mutations in *PRKAG2*,[7-9] the regulatory γ subunit of AMP-activated protein kinase. *PRKAG2* mutations cause myocyte hypertrophy by stimulating glycogen-filled vacuoles but cause neither myocyte disarray nor interstitial fibrosis, which typically occur with defects of sarcomere-protein genes.[9,10]

Pathologic vacuoles containing glycogen or intermediary metabolites also occur in Pompe's disease (a recessively inherited lysosomal acid α-1, 4-glucosidase [GAA] deficiency), Danon's disease (an X-linked lysosome-associated membrane protein [LAMP2] deficiency), and Fabry's disease (an X-linked lysosomal hydrolase a-galactosidase A [GLA] deficiency).[11-16] These multisystem disorders cause neuromuscular deficits, abnormal liver and kidney function, and abnormalities of the central nervous system as well as cardiac hypertrophy. Although some, atypical, patients with Fabry's disease have mild systemic manifestations and, predominantly, cardiac disease,[11,17] the pleiotropic manifestations of Pompe's disease and Danon's disease rarely prompt the consideration of these disorders in the differential diagnosis of unexplained left ventricular hypertrophy.

We sequenced eight sarcomere-protein genes in 75 unrelated patients with hypertrophic cardiomyopathy in whom echocardiography showed unexplained left ventricular hypertrophy. Subsequent analyses of *PRKAG2*, *LAMP2*, *GAA,* and *GLA* in samples of patients who did not have a sarcomere-protein gene mutation revealed previously unidentified *LAMP2* and *PRKAG2* mutations. The clinical manifestations associated with these mutations prompted studies of two additional patient series: one involved subjects with massive hypertrophy, and one involved those with left ventricular hypertrophy plus electrophysiological defects.

METHODS

CLINICAL EVALUATIONS

Studies were performed in accordance with institutional guidelines for human research. The research protocol was reviewed and approved by the institutional review boards at the participating institutions, and written informed consent was obtained from all research subjects. Three independent series of patients were studied: one involving 75 consecutive subjects 12 to 75 years of age who had hypertrophic cardiomyopathy as diagnosed on the basis of echocardiograms showing unexplained left ventricular hypertrophy (wall thickness, ≥13 mm)[3,18]; one involving 20 subjects (9 to 58 years of age) with massive left ventricular hypertrophy (wall thickness, ≥30 mm) of unknown cause; and one involving 24 patients (8 to 42 years of age) with hypertrophic cardiomyopathy in whom electrocardiograms suggested the presence of ventricular preexcitation (a short PR interval, delta wave, or both).

Study subjects were from North America, South America, and Europe and identified themselves as white (86 percent), black (7 percent), or Hispanic (7 percent). Medical records, clinical evaluations, electrocardiograms, and echocardiograms were reviewed. Clinical studies that were performed before enrollment at the discretion of the referring cardiologist were included when available. After completion of genetic studies, cardiac evaluations were performed of family members carrying a mutation. Patients with *LAMP2* mutations also underwent noninvasive neurologic and musculoskeletal evaluations and serum chemistry analyses. When available,

pathological specimens were examined. All values are reported as means ±SD.

GENETIC STUDIES

The genes encoding cardiac β-myosin heavy chain, cardiac myosin-binding protein C, cardiac troponin T, cardiac troponin I, cardiac actin, essential myosin light chain, regulatory myosin light chain, α-tropomyosin, and *PRKAG2* were sequenced from genomic DNA as described previously.[9,18] Exons 1 through 8, 9a, and 9b of *LAMP2*, exons 2 through 20 of *GAA*, and exons 1 through 7 of *GLA* were amplified with the use of the polymerase chain reaction (PCR) and sequenced and compared with GenBank accession numbers AC002476, NT_024915, and AL035422 with the use of primers available on the Internet (at http://genetics.med.harvard.edu/~seidman/). Sequence variants were confirmed by restriction-enzyme digestion. Variants that segregated with clinical status in family members and that were absent from 180 normal subjects who were matched with the subjects with hypertrophic cardiomyopathy for race or ethnic background (self-reported) were considered disease-causing mutations[18,19] and were denoted by standard nomenclature.[20] *LAMP2* alleles were distinguished by single-nucleotide polymorphisms 156 A/T and 927 C/T, numbered according to complementary DNA (cDNA) (GenBank accession number NM_013995).

RNA was extracted with the use of Trizol (Invitrogen). We performed reverse transcription (RT) using a kit (One-Step RT-PCR, Qiagen) with primers available on the Internet (at http://genetics.med.harvard.edu/~seidman/).

PROTEIN ANALYSES

Western blot analyses were performed in immunoprecipitation assay buffer with the use of 30 to 40 μg of protein lysates from lymphocytes or fibroblasts, as described previously,[21] with polyclonal LAMP2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies (Santa Cruz Biotechnology).

HISTOPATHOLOGICAL ANALYSES

Specimens were examined after staining with hematoxylin and eosin and LAMP2 immunohistochemistry. Electron-microscopical examinations of embedded tissue were performed after paraffin removal with the use of previously described procedures.[9]
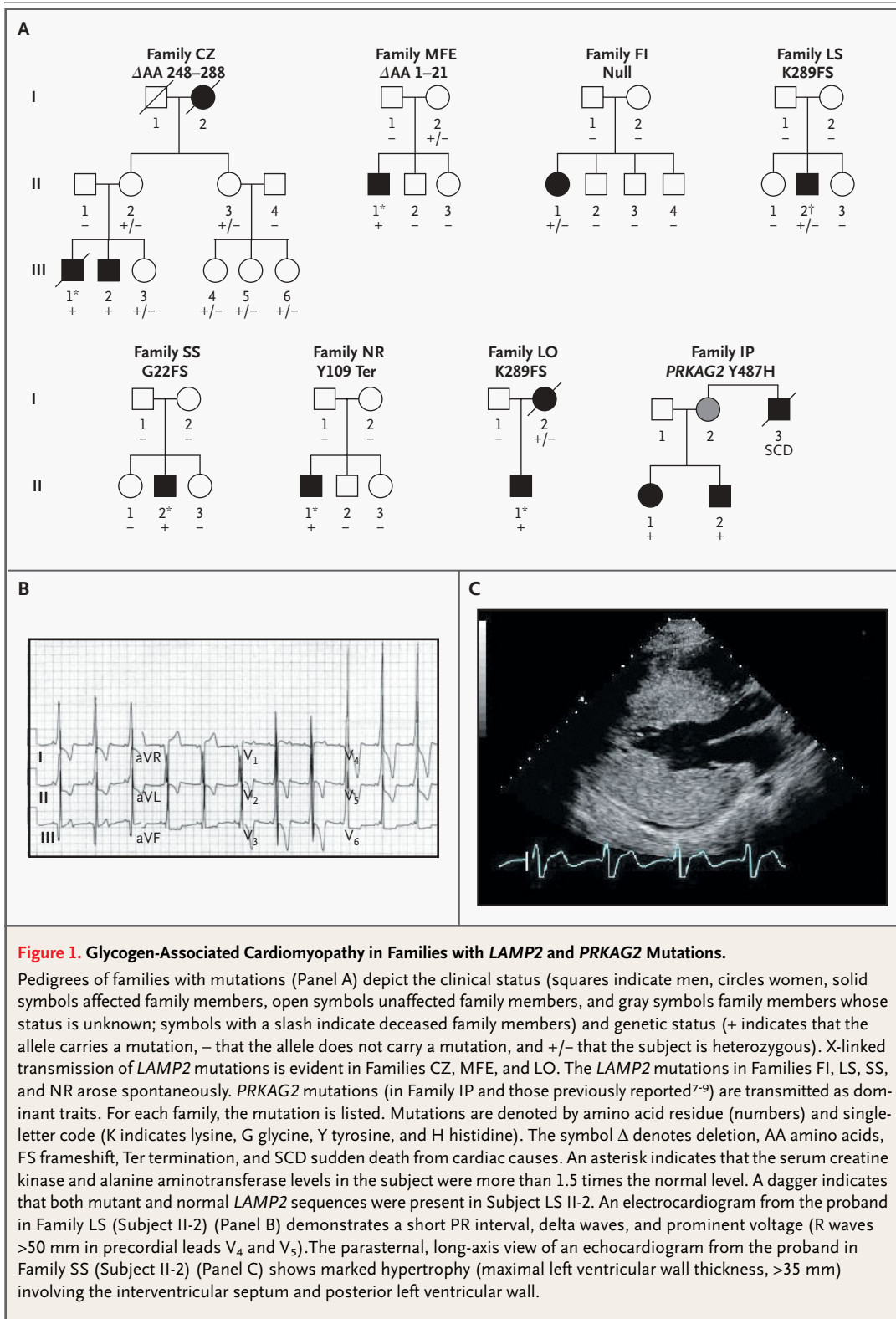
ELECTROCARDIOGRAPHY

Standard 12-lead electrocardiographic recordings were examined for ventricular preexcitation and left ventricular voltage (reported as the maximal S wave in V_1 or V_2 + maximal R wave in V_5 or V_6 [S_{V1} or S_{V2} + R_{V5} or R_{V6}] or the maximal R or S deflection in any lead[22]).

RESULTS

Seventy-five unrelated patients with hypertrophic cardiomyopathy (30 female and 45 male patients, 12 to 75 years of age at diagnosis) were prospectively enrolled for genetic analyses of sarcomere-protein mutations. Maximal left ventricular wall thickness ranged from 13 to 60 mm and in four persons exceeded 30 mm. In addition to typical electrocardiographic manifestations of hypertrophic cardiomyopathy,[3,18] three persons had short PR intervals and three others had ventricular preexcitation patterns. Forty sarcomere-protein gene mutations were identified in these 75 patients.[6,23] In the remaining 35 patients (27 of whom were male and 8 female), *PRKAG2*, *LAMP2*, *GLA*, and *GAA* sequences were analyzed. No mutations were identified in *GLA* or *GAA* sequences.

A previously undetected missense mutation — tyrosine to histidine at codon 487 — of *PRKAG2* in Proband IP was associated with moderate hypertrophy (left ventricular wall thickness, 13 mm) and an extremely short PR interval (0.09 msec). *LAMP2* mutations in which the sequence GTGA was deleted from the splice-donor site of intron 6 (IVS6+1_4delGTGA) and in which there was an A-to-G change in the splice-acceptor site of intron 6 (IVS6−2A→G) in Probands CZ and FI were associated with severe hypertrophy (left ventricular wall thicknesses, 29 mm and 60 mm, respectively) and unusual electrocardiograms, with short PR intervals, short delta waves, or both, and extreme voltage, suggestive of ventricular preexcitation (Fig. 1). These clinical findings prompted the genetic studies of two additional patient series. *LAMP2* and *PRKAG2* sequences were determined in 20 patients with a left ventricular wall thickness of 30 mm or more; however, no mutations were identified. *LAMP2* and *PRKAG2* sequences were also determined in 24 probands with increased left ventricular wall thickness and electrocardiograms suggesting ventricular preexcitation. Seven *PRKAG2*

Figure 1. Glycogen-Associated Cardiomyopathy in Families with *LAMP2* and *PRKAG2* Mutations.

Pedigrees of families with mutations (Panel A) depict the clinical status (squares indicate men, circles women, solid symbols affected family members, open symbols unaffected family members, and gray symbols family members whose status is unknown; symbols with a slash indicate deceased family members) and genetic status (+ indicates that the allele carries a mutation, – that the allele does not carry a mutation, and +/– that the subject is heterozygous). X-linked transmission of *LAMP2* mutations is evident in Families CZ, MFE, and LO. The *LAMP2* mutations in Families FI, LS, SS, and NR arose spontaneously. *PRKAG2* mutations (in Family IP and those previously reported[7-9]) are transmitted as dominant traits. For each family, the mutation is listed. Mutations are denoted by amino acid residue (numbers) and single-letter code (K indicates lysine, G glycine, Y tyrosine, and H histidine). The symbol Δ denotes deletion, AA amino acids, FS frameshift, Ter termination, and SCD sudden death from cardiac causes. An asterisk indicates that the serum creatine kinase and alanine aminotransferase levels in the subject were more than 1.5 times the normal level. A dagger indicates that both mutant and normal *LAMP2* sequences were present in Subject LS II-2. An electrocardiogram from the proband in Family LS (Subject II-2) (Panel B) demonstrates a short PR interval, delta waves, and prominent voltage (R waves >50 mm in precordial leads V_4 and V_5). The parasternal, long-axis view of an echocardiogram from the proband in Family SS (Subject II-2) (Panel C) shows marked hypertrophy (maximal left ventricular wall thickness, >35 mm) involving the interventricular septum and posterior left ventricular wall.

mutations[9] and four *LAMP2* mutations were identified, which corresponded to a mutation-detection rate of 46 percent in this group.

LAMP2 MUTATIONS

Six new *LAMP2* mutations were detected — in Probands CZ, IP, LS, MFE, NR, and SS — that are predicted to alter substantially the lysosome-associated membrane protein, a 410-amino-acid molecule with a small cytoplasmic tail involved in receptor-mediated lysosomal uptake, and a large internal lysosome domain composed of highly glycosylated residues. One nonsense mutation (in Proband NR) signaled premature termination at amino acid 109. Five other mutations altered splice signals; the consequences of these alterations were assessed in the *LAMP2* RNAs isolated from lymphocytes.

Mutation IVS1+1G→T (in Proband MFE) altered the intron 1 splice donor site; RNA maturation occurred by a cryptic splice site that excised 21 amino acids after the initiation codon. Mutation IVS1−2A→G (in Proband SS) altered the intron 1 splice-acceptor site; RNA deleted exon 2 residues and produced a frameshift mutation. Mutation IVS6+1_4delGTGA (in Proband CZ) altered the intron 6 splice-donor site and excised 41 codons. No mutant RNA was detected from mutation IVS6−2A→G (in Proband FI) that altered the splice-acceptor site of intron 6, perhaps indicating that this defect triggered missense-mediated decay. Mutation 928G→A (in Proband LS) substituted isoleucine for valine (at residue 310), affected RNA processing, and hence produced a frameshift.

Expression of the mutant LAMP2 protein was assessed by Western blotting of protein extracts probed with antibodies to LAMP2 (Fig. 2) and antibodies to GAPDH (data not shown). Protein extracts from the lymphocytes of Probands MFE and CZ (mutations IVS1+1G→T and IVS6+1_4delGTGA, respectively) contained a nearly full-length LAMP2 protein (100 kD), whereas protein extract from the lymphocytes and fibroblasts of Proband SS (mutation IVS1−2A→G) did not react with LAMP2 antibodies.

CLINICAL FEATURES IN PROBANDS
WITH *LAMP2* MUTATIONS

Five of six probands with *LAMP2* mutations were male. One proband had a family history of heart disease. None had mental retardation or overt neurologic or musculoskeletal deficits. Two male probands had histories of attention-deficit disorder and mild behavioral problems; both were taking psychoactive medications.

One asymptomatic proband came to medical attention because of an abnormal electrocardiogram. The other five probands presented with cardiac symptoms typically seen in hypertrophic cardiomyopathy, including chest pain, palpitations, syncope, and, in one, cardiac arrest. The onset of symptoms occurred between the ages of 8 and 15 years, younger than average for patients with mutations of the sarcomere-protein gene or *PRKAG2* gene (33±17 years and 31±15 years, respectively) (Table 1).

Echocardiography showed concentric left ventricular hypertrophy in all six probands; in five, left ventricular hypertrophy was massive. The average maximal left ventricular wall thickness was 35±15 mm (range, 20 to 60 mm) and significantly greater (P<0.01) than that typically found in patients with hypertrophic cardiomyopathy that is diagnosed on the basis of either clinical findings (average, 21 mm[24]) or genetic analyses (Table 1). Two probands (NR and SS) had substantial outflow tract gradients (55 and 65 mm Hg, respectively). Prominent right ventricular hypertrophy (wall thickness, ≥10 mm) was found without pulmonary disease in three probands. At the time of initial clinical presentation, all probands had normal left ventricular function and ejection fractions of 60 percent or more.

Twelve-lead electrocardiograms were strikingly abnormal in all probands. Left ventricular voltage was markedly increased and significantly greater (P<0.001) than in patients with sarcomere-protein gene mutations or *PRKAG2* mutations (Table 1). In five probands, electrocardiograms showed ventricular preexcitation patterns with short PR intervals and delta waves (Fig. 1B). Electrophysiological studies in three persons showed accessory atrioventricular connections; two had supraventricular arrhythmias, atrial fibrillation, or both, that required radiofrequency ablation.

The identification of *LAMP2* mutations prompted analyses of serum chemistry. Creatine kinase and alanine aminotransferase levels were elevated by a factor of two or more in four of the six probands, and organ-specific enzyme isoforms indicated cardiac, musculoskeletal, and liver involvement. Serum levels of these enzymes were normal in the only female proband and in one male proband with a mosaic *LAMP2* mutation.

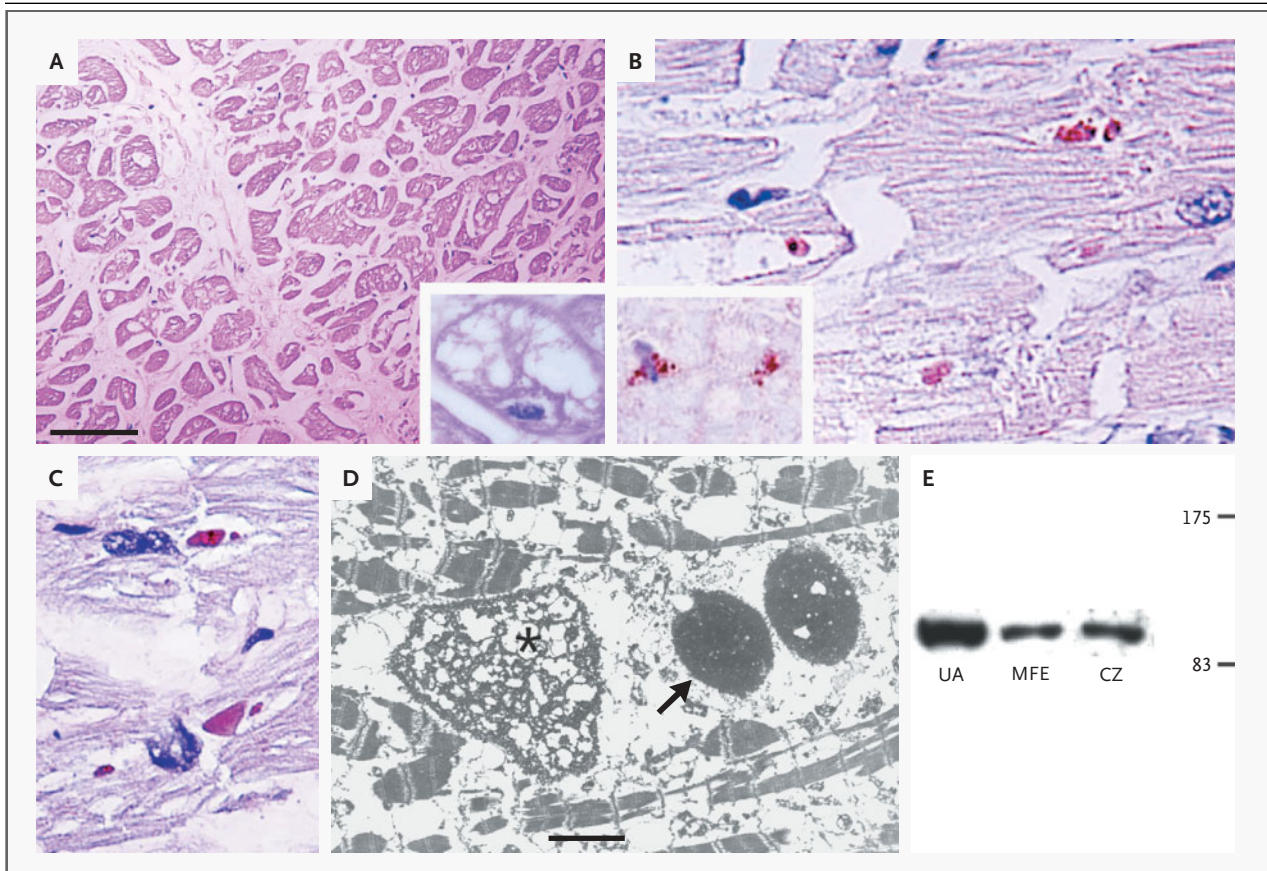Cardiac function progressively deteriorated during a six-year period in Family Member CZ III-1,[25]

Figure 2. Histopathological Findings in Cardiac Tissue from a Patient with a *LAMP2* Mutation.

A light micrograph of cardiac tissue from Proband CZ (Panel A) shows diffusely enlarged cardiomyocytes with prominent cytoplasm, pleomorphic nuclei, and numerous cytoplasmic vacuoles. A vacuolated myocyte with a "spider cell" (inset) resembles rhabdomyoma cells (hematoxylin and eosin; the bar represents 100 μm). Immunohistochemical analyses with LAMP2-specific antibodies reveals positive (red) staining within vacuoles (Panel B). In normal myocardium, strong granular perinuclear staining of lysosomes is evident (inset). Vacuoles (Panel C), containing large periodic acid–Schiff–positive inclusions, are homogeneous, with well-defined borders. An electron micrograph of myocytes (Panel D) shows large, densely osmophilic perinuclear inclusions (arrow) containing fibrillogranular material with variable density and an absence of visible membranes. The nucleus is poorly preserved (asterisk). The bar represents 2 μm. Western blotting (Panel E) detected LAMP2 protein in lymphocyte extracts of unaffected (UA) control samples and samples from Probands CZ and MFE. The mobility of 83 and 175 kD is indicated.

and he died at the age of 22 years while awaiting heart transplantation. A pathological study of his heart (Fig. 2A through 2D) showed marked cardiomegaly (weight, 1266 g) and diffuse hypertrophy. Histopathological examination showed myocyte hypertrophy and prominent interstitial fibrosis. Enlarged cardiomyocytes had extensive sarcoplasmic vacuolation with a spiderweb-like appearance; some had large, polymorphic, periodic acid–Schiff–positive perinuclear inclusions. LAMP2 antibodies reacted with the inclusions but lacked the lysosomal perinuclear granular pattern found in normal myocardium. Electron microscopy showed that some

sarcoplasmic vacuoles were empty, without recognizable membranes, whereas other vacuoles contained inclusions consisting of amorphous, osmophilic, and focally granular material of variable density. Partially degraded vacuolar contents have been observed in specimens from persons with Danon's disease.[12,15]

LAMP2 MUTATIONS IN FAMILY MEMBERS

In Families CZ and MFE, the X-linked *LAMP2* mutation was maternally transmitted; one additional man (Family Member CZ III-2) and seven women also carried these *LAMP2* mutations (Fig. 1). Cardiac dis-

Table 1. Cardiac Findings Associated with Mutations in Sarcomere-Protein Genes, *PRKAG2,* and *LAMP2.**

Variable	Sarcomere-Protein Genes (N=40)†‡	*PRKAG2* (N=32)‡§	*LAMP2* (N=7)‡
Age at diagnosis (yr)	33±17¶	31±15¶	15±4
No. of distinct mutations	35	4	6
Preexcitation (%)	0¶	9 (28)‖	6 (86)
Maximal left ventricular wall thickness (mm)	24±10¶	17±8¶	35±15
S_{V1} or S_{V2} + R_{V5} or R_{V6}	48±21¶	40±21‖	92±46
Maximal R or S (mV)	34±13¶	32±11¶	67±17

* All statistical analyses are comparisons with data on *LAMP2.* Measurements of clinical parameters were not statistically different among subjects with the *PRKAG2* mutation with or without ventricular preexcitation. Statistical comparisons of preexcitation were calculated with the use of the chi-square test; all others were calculated with the use of the Wilcoxon rank-sum test. Plus–minus values are means ±SD.
† Subjects with hypertrophic cardiomyopathy had a defined sarcomere-protein gene mutation.
‡ Numbers of subjects include probands and clinically affected family members with electrocardiograms showing intrinsic rhythm.
§ Data from *PRKAG2* mutations include patients identified in this study and in a previously reported cohort.[9]
¶ P<0.002.
‖ P<0.01.

ease occurred only in Family Member CZ III-2. When he was 16 years old, his electrocardiogram showed prominent voltage and ventricular preexcitation. His echocardiogram from the same period was normal except for asynchronous contraction, which was attributed to preexcitation. Reevaluation at the age of 22 (after genetic diagnosis) demonstrated substantial hypertrophy and reduced function. He remains asymptomatic.

Although none of seven surviving female family members (14 to 46 years of age) with *LAMP2* mutations had cardiac symptoms or abnormal cardiac studies, one woman (Family Member CZ I-2) died from congestive heart failure at the age of 44. She probably carried the *LAMP2* mutation, given that both of her daughters are genetically affected and that her husband does not have cardiac disease (Family Member CZ-1).

No *LAMP2* mutations were found in the family members of four probands. *LAMP2* gene sequences were normal in the mothers of three affected male probands (SS, LS, and NR), and mitochondrial DNA polymorphisms confirmed biologic maternity (data not shown). Genetic studies of Proband LS demonstrated mosaicism: both mutant and wild-type

LAMP2 sequences were identified despite a normal XY karyotype (not shown). Single nucleotide polymorphisms indicated that he had inherited the X chromosome and normal *LAMP2* gene from his unaffected mother. One female proband (FI) with a normal karyotype also carried a *LAMP2* mutation. Haplotype analyses (not shown) demonstrated that she had inherited one X chromosome from each genetically unaffected parent. We conclude that these *LAMP2* mutations (in four probands: SS, LS, NR, and FI) arose spontaneously.

DISCUSSION

Defects in the enzymes involved in the metabolism of muscle glycogen typically cause systemic disease[26] and often involve the heart (Fig. 3). Our study demonstrates that cardiac disease can be the initial and predominant manifestation of defects in human glycogen metabolism. Three of 75 persons in whom hypertrophic cardiomyopathy was diagnosed by echocardiography had cardiac-glycogen–storage disorders caused by *LAMP2* or *PRKAG2* mutations. These gene defects, like sarcomere-gene mutations, were associated with prominent left ventricular hypertrophy, but in addition, electrophysiological abnormalities were present.

Family history, although informative in terms of sarcomere-protein gene and *PRKAG2* mutations, was typically absent for patients with *LAMP2* defects; these defects cause sporadic disease. Male sex, early onset of symptoms, marked or massive concentric left ventricular hypertrophy, prominent electrocardiographic voltages with ventricular preexcitation patterns (Fig. 1), and asymptomatic elevations of serum-chemistry values further distinguished *LAMP2* mutations from *PRKAG2* or sarcomere-protein defects.

Previously reported *LAMP2* mutations caused a variety of manifestations that are characteristic of Danon's disease.[12-14] No probands in our series had clinically important neurologic disease, although psychological issues recognized in two young male subjects were attributed to attention-deficit disorder and an adolescent response to cardiac disease. None had overt muscle weakness, wasting, or myopathic symptoms; all had exercise restrictions because of the diagnosis of hypertrophic cardiomyopathy.

Gene dosage probably accounts for the different clinical consequences of X-linked *LAMP2* mutations in men as compared with women, although
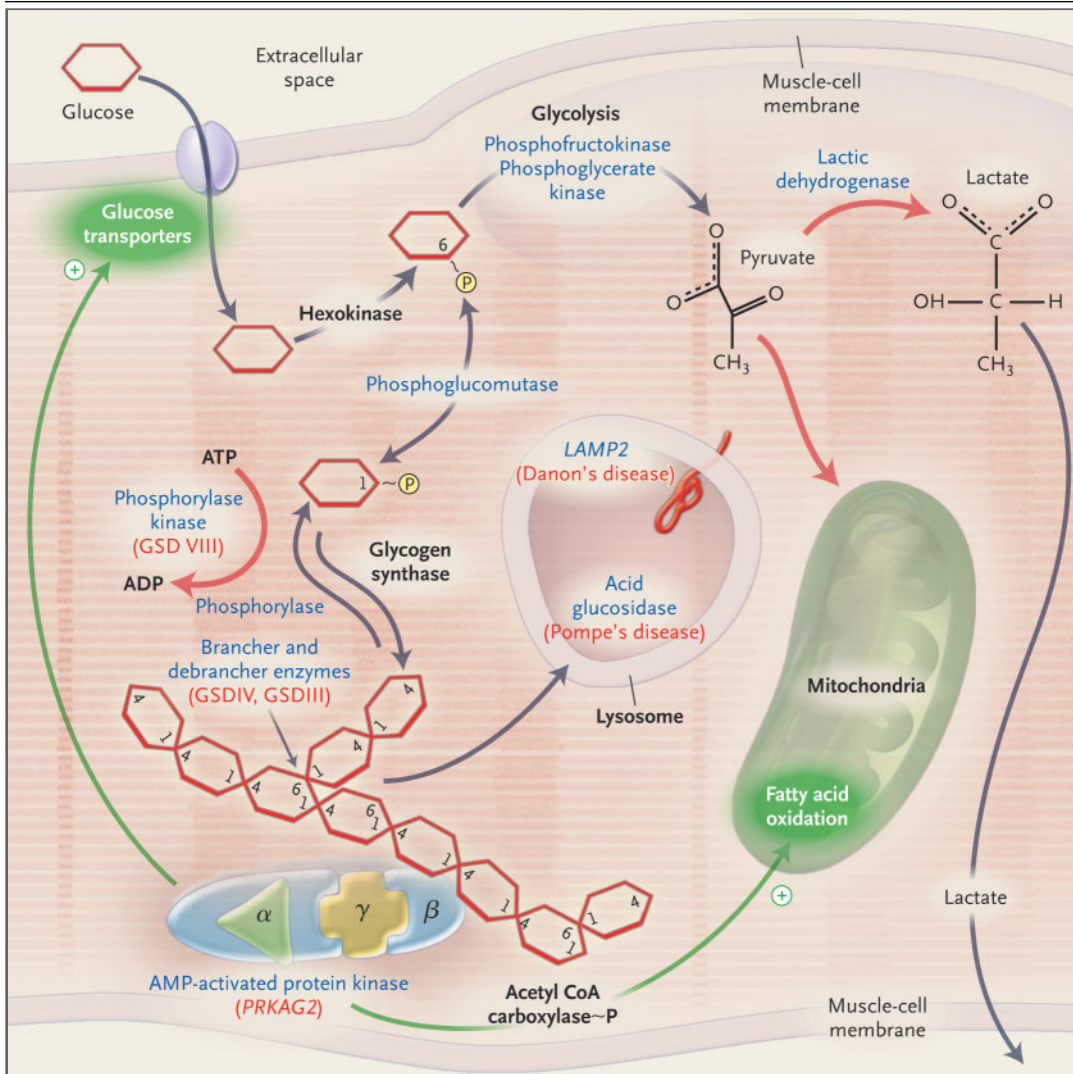
Figure 3. Principal Pathways of Glycogen Metabolism in Muscle.

Proteins (blue lettering) involved in glycogen storage diseases (GSDs) associated with cardiomyopathy (red lettering) are shown. Glucose enters muscle cells through transport proteins and undergoes phosphorylation by hexokinase, after which it is targeted for glycolysis or glycogen synthesis by glycogen synthase. Glycogen, a branched glucose polymer containing 93 percent 1–4 glucose bonds and 7 percent branched 1–6 glucose bonds, is a dynamic reservoir of energy for muscles; synthesis or degradation depends on the activity of specific enzymes that undergo reversible phosphorylation by kinases. Glycogen metabolism is further influenced by AMP-activated protein kinase, which associates with glycogen and regulates glucose uptake, and by lysosome activity. Defects in glycogen-degradation pathways (involving phosphorylase, phosphorylase kinase, phosphoglucomutase, phosphofructokinase, phosphoglycerate kinase, lactic dehydrogenase, and brancher and debrancher enzymes) result in glycogen accumulation and exercise-induced skeletal muscle symptoms and myoglobinuria, with or without cardiac manifestations. AMPK, which consists of α, γ, and β subunits, also regulates fatty acid oxidation through phosphorylation of acetyl CoA carboxylase (acetyl CoA carboxylase~P). Defects in *PRKAG2* (the regulatory γ subunit of AMPK), *LAMP2* or acid glucosidase cause insidious glycogen accumulation, resulting in cardiac hypertrophy and electrophysiological abnormalities.

unusual cardiac diseases were found in two female carriers of *LAMP2* mutations. Perhaps X-inactivation sufficiently extinguished normal *LAMP2* gene expression to contribute to or cause cardiomyopathy in female Proband FI and adult-onset heart failure in Family Members CZ I-2 and LO I-2 (Fig. 1A). Gene dosage also contributed to clinical expression in men with identical *LAMP2* mutations. During these studies, a male proband (LO in Fig. 1A) with classical Danon's disease (mental retarda-

tion and musculoskeletal weakness, with protean findings on muscle biopsy) was referred for genetic analyses. Proband LO and his mother were found to have the same *LAMP2* mutation (928G→A) as Proband LS, although these families are genetically unrelated (data not shown). Remarkably, Proband LO was hemizygous for the mutation, whereas mosaicism in Proband LS caused expression of both normal and mutant *LAMP2* alleles. We presume that some normal LAMP2 protein in Proband LS
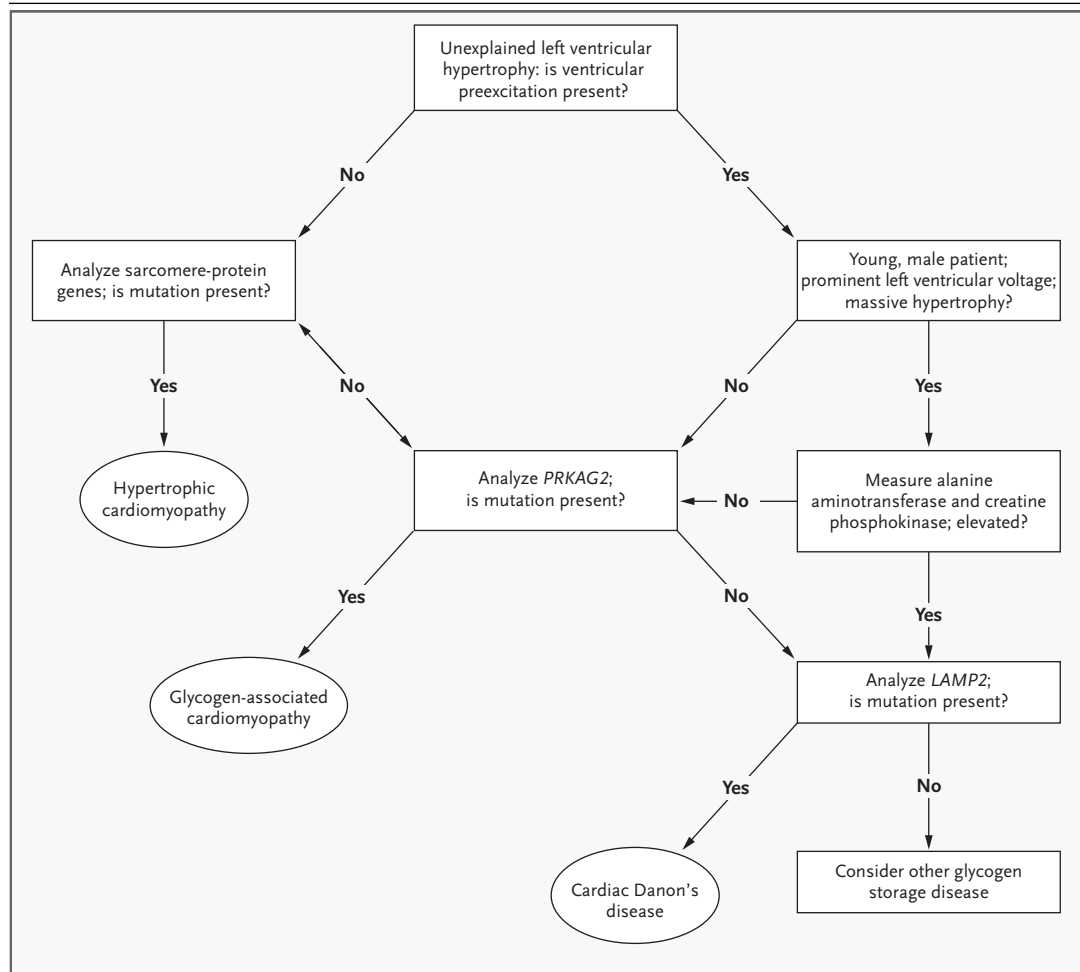

Figure 4. Algorithm for the Diagnostic Evaluation of Persons with Unexplained Left Ventricular Hypertrophy.
A family history of the dominant inheritance of left ventricular hypertrophy, unaccompanied by systemic manifestations or electrocardiographic findings of ventricular preexcitation, suggests hypertrophic cardiomyopathy; the identification of a sarcomere mutation confirms the diagnosis. In young patients with echocardiographic findings of unexplained left ventricular hypertrophy and electrocardiograms with prominent left ventricular voltage and short PR intervals or delta waves, or both, glycogen storage disease should be suspected. Dominant inheritance and an absence of systemic disease suggest the presence of glycogen-associated cardiomyopathy due to *PRKAG2* mutations. Male sex and abnormalities in liver, musculoskeletal, or neurologic function suggest a diagnosis of Danon's disease, although systemic manifestations can be modest or absent in the cardiac form of this disease. When the cause is not established by genetic analyses, a tissue biopsy and a biochemical study may be helpful.

accounted for the predominance of cardiac disease in comparison with multisystem Danon's disease in Proband LO.

The partial function of mutant LAMP2 proteins may also account for the cardiac form of Danon's disease, as compared with systemic Danon's disease. The musculoskeletal pathology of Danon's disease[13,14] indicates a complete absence of *LAMP2* immunoreactivity, whereas we found stable *LAMP2* RNA and immunoreactive LAMP2 protein in lymphocytes from Probands CZ and MFE (Fig. 2E). These mutant proteins may function sufficiently to limit disease in some, but not all, tissues.

Inclusion of *LAMP2* and *PRKAG2* mutations in the differential diagnosis of unexplained left ventricular hypertrophy is important for patient care. These mutations increase the risk of arrhythmias, as shown by preexcitation patterns on electrocardiograms, by accessory pathways on electrophysiological evaluation, and by patients' histories of supraventricular tachyarrhythmias, syncopal episodes, and sudden death. The mechanism for ventricular preexcitation is incompletely understood; however, a mouse model of one human *PRKAG2* mutation shows disruption of the anulus fibrosus by glycogen-filled myocytes, thereby allowing atrioventricular activation that bypasses the atrioventricular node.[10,27] Although *LAMP2* mutations accumulate glycogen in lysosomes[12] and *PRKAG2* mutations accumulate glycogen throughout the myocyte,[10] it is likely that there is a common mechanism for ventricular preexcitation in both glycogen-storage cardiomyopathies. We suggest that patients with unexplained left ventricular hypertrophy and preexcitation patterns on electrocardiograms undergo clinical and genetic evaluation for glycogen storage disease (Fig. 4).

The different clinical courses associated with hypertrophic cardiomyopathy or glycogen storage cardiomyopathies underscore the importance of accurate diagnosis. Despite some increase in the risk of sudden death in patients with hypertrophic cardiomyopathy, the natural history of and treatment for sarcomere mutations are generally favorable: symptoms typically develop in early adulthood and increase slowly over many years; interventions that either alleviate outflow-tract obstruction or terminate arrhythmias, or both, improve long-term survival[2,3,28]; and progression to heart failure is uncommon (occurring in fewer than 10 percent of patients). Cardiomyopathy due to *PRKAG2* mutations is also compatible with long-term survival, although progressive conduction-system disease may necessitate the implantation of a pacemaker and aggressive control of arrhythmias.[7-9] By contrast, the prognosis associated with cardiomyopathy due to *LAMP2* mutations is poor. The onset of disease during adolescence is followed by a rapid progression toward end-stage heart failure early in adulthood, often resulting in death.[13,14]

Although clinical evaluations may help to distinguish these disorders, genetic analyses can definitively establish the cause of unexplained left ventricular hypertrophy (Fig. 4). This information is critical for determining the appropriate strategies of treatment and for defining genetic risk in family members. Applying the major advances in DNA sequencing to medicine has made gene-based diagnosis not only feasible, but a clinical reality.

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