Dilated cardiomyopathy (DCM) is inherited commonly as a dominant disorder associated with a range of genetic defects (1). Despite genetic heterogeneity, abnormal calcium handling is a hallmark of DCM (2). An increase in cytosolic calcium or prolonged intervals of increased calcium produces cardiomyocyte dysfunction and predisposes to arrhythmias (3). The sarcoplasmic reticulum calcium ATPase 2a (SERCA2a) mediates cyclic uptake of calcium from the myoplasm into the sarcoplasmic reticulum; SERCA2a is regulated, in part, by phospholamban, a small 52 amino acid membrane-associated protein (4). With dephosphorylation, phospholamban inhibits SERCA2a leading to prolongation of increased cytosolic calcium. Phospholamban is phosphorylated by protein kinase A (PKA) leading to derepression, or activation, of SERCA2a and an increase in removal of cytosolic calcium during diastole.

Consistent with the importance of calcium handling to normal myocyte function, phospholamban gene (PLN) mutations have been found in inherited DCM (5,6). A point mutation in PLN, R9C, was found in a single large family with an average age of onset of 20 to 30 years and sudden death at an average of 25 (5). Transgenic mice expressing PLN R9C under the control of the alpha myosin heavy chain gene promoter developed lethal cardiomyopathy. The PLN R9C mutation sequesters PKA preventing phosphorylation of normal phospholamban and enhanced inhibition of SERCA2a activity (5). A second mutation, L39X, was also reported linked to human DCM (6). In this case, when carried heterozygously, the L39X mutation produced an asymptomatic hypertrophic cardiomyopathy. In the homozygous state, the L39X mutation was associated with early-onset, lethal, DCM. Most recently, a deletion of R14 in phospholamban (PLN R14del) was found in a large family with early-onset lethal DCM (7). This mutation was also shown to sequester PKA leading to a decrease in phospholamban phosphorylation and SERCA2a inhibition.

We now screened a cohort of 260 DCM patients for mutations in the PLN. We found a single family with 2 affected individuals. We independently identified these patients as carrying the PLN R14del mutation. Strikingly, the age of onset in these patients was in the seventh decade. Therefore, mutations in phospholamban can be associated with a variable phenotype including late-onset, mild cardiomyopathy.

METHODS

Human subjects. Criteria for inclusion included: 1) ejection fraction <45% or 2) left ventricular (LV) end diastolic diameter >117% of the predicted value, and 3) absence of significant epicardial coronary artery disease, and/or hypertensive, systemic, pericardial, or congenital disease that explains the cause of DCM (8,9). Written and informed consent was obtained in accordance with the University of Chicago's Institutional Review Board. Clinical data were obtained through evaluations performed at the University of Chicago Cardiology Clinics. Family history was ascertained from the patients by a certified genetic counselor, and
Abbreviations and Acronyms

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>DCM</td>
<td>dilated cardiomyopathy</td>
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<tr>
<td>LV</td>
<td>left ventricle/ventricular</td>
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<tr>
<td>PLN</td>
<td>phospholamban gene</td>
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<tr>
<td>PLN R14del</td>
<td>deletion of R14 in phospholamban</td>
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<tr>
<td>PKA</td>
<td>protein kinase A</td>
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<tr>
<td>SERCA2a</td>
<td>sarcoplasmic reticulum calcium ATPase 2a</td>
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Medical records were obtained from family members after proband to family member contact.

**Genetic analysis.** Deoxyribonucleic acid was isolated from whole blood using PureGene (Gentra Systems, Inc., Minneapolis, Minnesota) according to recommendations. Direct sequencing of polymer chain reaction products was completed using cycle sequencing and compared with the human PLN sequence (GenBank Accession NM_002667). Single strand conformation polymorphism analysis was performed using MDE (FMC Corporation, Philadelphia, Pennsylvania) as described (10). Structural modeling was performed using Cn3D 4.1.

**RESULTS**

**DCM cohort.** Of the 260 unrelated DCM subjects screened, 35% of these had a strong family history of DCM as defined by at least 1 relative with proven DCM. An additional 27% had a “suspicious” or moderately positive family history for DCM because of sudden death at a young age in a first-degree relative (female subjects <50 years of age, male subjects <45 years of age), and the circumstances surrounding the death were suspicious for arrhythmia. Thirty-seven percent did not have a family history suggestive of DCM and/or arrhythmias, and family history was not available for 1% of subjects.

**Clinical and genetic results.** We identified a single PLN mutation, R14del, in a 61-year-old Caucasian woman (DCM-X1) initially evaluated in a muscular dystrophy clinic for a 25-year history of slowly progressive muscle weakness. She noted leg pain, difficulty standing from a sitting position, and difficulty climbing stairs. Neurological exam showed mild weakness in hip flexion and abduction with normal strength in other limb muscles. A quadriceps skeletal muscle biopsy showed no significant abnormalities with normal staining patterns for dystrophin, α-sarcoglycan, β-sarcoglycan, γ-sarcoglycan, merosin, α-dystroglycan, β-dystroglycan, and dysferlin. Her serum creatine kinase was normal. An echocardiogram revealed severely impaired LV performance with an estimated ejection fraction of 20%, 4-chamber dilatation, and impaired right ventricular function. Cardiac catheterization revealed no significant coronary artery disease and an apical LV thrombus. At age 63, the patient underwent prophylactic implantation of a defibrillator. The patient had 4 successful pregnancies in her third and fourth decades.

The proband’s family history was significant for her brother (DCM-X2) who was found at age 70 to have an LV ejection fraction of 15% to 20% (Fig. 1). He also was found to have the PLN R14del mutation. He was active with no complaints related to heart failure. During his evaluation, he was noted to have atrial flutter and bradycardia and no significant coronary artery disease. He received a single-chamber atrial pacemaker that was upgraded to a dual-chamber defibrillator at age 72 after a syncopal episode. Other members of the family do not carry PLN R14del and have normal echocardiograms. The mutation was not detected in 100 unrelated normal, ethnically matched control subjects.

**DISCUSSION**

The PLN mutation in this family falls within a conserved domain of phospholamban (Fig. 2). It cannot be determined whether R13 or R14 is deleted because the codon for each

![Figure 1. Phospholamban gene R14del in dilated cardiomyopathy. (A) Pedigree with proband (*). + or − indicates the presence or absence of the PLN R14del mutation. (B) Single-strand conformation change indicating the phospholamban gene R14 deletion mutation in the members of the pedigree. Lanes 10 and 11 represent the proband and her brother, respectively.](image-url)
Mal lives with no decrement in symptomatic cardiac function with asymptomatic DCM. Before diagnosis, both led normally.

A family in which 2 individuals carry the identical PLN R14del, yet these individuals display a distinctly milder form of early-onset familial DCM (7). This severe phenotype resembles the R9C mutation and displays a similar outcome of PLN-mediated cardiomyopathy. Several studies have suggested that the level of phospholamban expression is critical in mediating its effect (4). It is possible that the mutant allele of PLN is expressed at a lower level leading to a smaller pathogenic effect in these individuals. Alternatively, mutations in genes outside of the PLN locus may be present that lead to a less severe phenotype in these individuals.

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**REFERENCES**