Lamin A/C-Related Cardiac Disease

Late Onset With a Variable and Mild Phenotype in a Large Cohort of Patients With the Lamin A/C p.(Arg331Gln) Founder Mutation

Edgar T. Hoorntje, MD; Ilse A. Bollen, MSc; Daniela Q. Barge-Schaapveld, MD, PhD; Florence H. van Tienen, PhD; Gerard J. te Meerman, PhD; Joeri A. Jansweijer, MD; Anthonie J. van Essen, MD, PhD; Paul G. Volders, MD, PhD; Alina A. Constantinescu, MD, PhD; Peter C. van den Akker, MD, PhD; Karin Y. van Spaendonck-Zwarts, MD, PhD; Rogier A. Oldenburg, MD, PhD; Carlo L. Marcelis, MD; Jasper J. van der Smagt, MD; Eric A. Hennekam; Aryan Vink, MD, PhD; Marianne Bootsma, MD, PhD; Emmelien Aten, MD, PhD; Arthur A. Wilde, MD, PhD; Arthur van den Wijngaard, PhD; Jos L. Broers, PhD; Jan D. Jongbloed, PhD; Jolanda van der Velden, PhD; Maarten P. van den Berg, MD, PhD; J. Peter van Tintelen, MD, PhD

Background—Interpretation of missense variants can be especially difficult when the variant is also found in control populations. This is what we encountered for the *LMNA* c.992G>A (p.(Arg331Gln)) variant. Therefore, to evaluate the effect of this variant, we combined an evaluation of clinical data with functional experiments and morphological studies.

Methods and Results—Clinical data of 23 probands and 35 family members carrying this variant were retrospectively collected. A time-to-event analysis was performed to compare the course of the disease with carriers of other *LMNA* mutations. Myocardial biopsies were studied with electron microscopy and by measuring force development of the sarcomeres. Morphology of the nuclear envelope was assessed with immunofluorescence on cultured fibroblasts. The phenotype in probands and family members was characterized by atrioventricular conduction disturbances (61% and 44%, respectively), supraventricular arrhythmias (69% and 52%, respectively), and dilated cardiomyopathy (74% and 14%, respectively). LMNA p.(Arg331Gln) carriers had a significantly better outcome regarding the composite end point (malignant ventricular arrhythmias, end-stage heart failure, or death) compared with carriers of other pathogenic *LMNA* mutations. A shared haplotype of 1 Mb around *LMNA* suggested a common founder. The combined logarithm of the odds score was 3.46. Force development in membrane-permeabilized cardiomyocytes was reduced because of decreased myofibril density. Structural nuclear *LMNA*-associated envelope abnormalities, that is, blebs, were confirmed by electron microscopy and immunofluorescence microscopy.

Conclusions—Clinical, morphological, functional, haplotype, and segregation data all indicate that LMNA p.(Arg331Gln) is a pathogenic founder mutation with a phenotype reminiscent of other *LMNA* mutations but with a more benign course. **(*Circ Cardiovasc Genet*. 2017;10:e001631. DOI: 10.1161/CIRCGENETICS.116.001631.)**

Key Words: atrial fibrillation ■ atrioventricular block ■ cardiomyopathy, dilated ■ lipodystrophy ■ survival

The *LMNA* gene encodes for the intermediate filament proteins lamin A and C. *LMNA* mutations are associated with a wide spectrum of phenotypes ranging from progeroid syndromes, muscular disease, and lipodystrophy to isolated cardiac disease (dilated cardiomyopathy [DCM] and conduction disorders) and phenotypes consisting of combinations of these different features.[1] Although their precise role is unknown, LMNA proteins are believed to play an important role in the structural integrity of the cell nucleus and in gene regulation.[2]

LMNA is one of the genes most frequently involved in genotyped DCM.[3] Sinus node dysfunction, atrioventricular conduction disorders, and supraventricular and ventricular arrhythmias often precede or accompany DCM.[4] *LMNA*-related cardiac disease is associated with a high incidence of major cardiac events like sudden cardiac death, appropriate implantable cardioverter-defibrillator (ICD) therapy, or end-stage heart failure. DCM patients with an *LMNA* mutation are, in general, believed to have a poor prognosis compared with non–*LMNA* mutation DCM patients.[5,6]

See Editorial by Arbustini
See Clinical Perspective

Currently, with all the new DNA-sequencing technologies implemented in routine patient care, increasing numbers

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Correspondence to J. Peter van Tintelen, MD, PhD, Department of Clinical Genetics, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. E-mail p.vantintelen@amc.nl

of DNA variants are being identified.[7] Classifying a variant as pathogenic has important implications for genetic counseling, the identification of family members at risk, clinical management, and sometimes even clinical risk stratification.[8] However, assigning pathogenicity to a variant is often challenging, particularly when the variant is found in ostensibly healthy controls.

In the Genome of the Netherlands (GoNL) database, which contains genome-sequencing data of ≈500 unrelated Dutch subjects, *LMNA* c.992G>A (p.(Arg331Gln)) was found 4 times but in low-quality calls. We, therefore, had difficulty assigning the correct label to this variant. Although in silico prediction models predicted that this substitution is deleterious or probably damaging, we sought to find additional evidence for the potential pathogenicity of this mutation by evaluating clinical data, studying morphology of the nuclear envelope, and analyzing functional effects on the myocytes and fibroblasts of mutation carriers.

Methods

Mutation Analysis

Probands with the LMNA p.(Arg331Gln) variant were identified by next-generation sequencing using targeted panels (the list of genes screened is available on request) or by Sanger sequencing.[9,10] Variants found with next-generation sequencing were confirmed by Sanger sequencing. Variants in family members were analyzed by Sanger sequencing. Written informed consent was obtained from all participants according to the local medical ethics committees of our hospitals.

The online GoNL database and the Exome Aggregation Consortium data set were searched for the LMNA p.(Arg331Gln) variant to check for its presence in the general population.[11,12]

Clinical Evaluation

We collected retrospective clinical data on 23 probands (A–W), who carried the LMNA p.(Arg331Gln) variant and on 35 family members carrying this variant. When available, data on medical history, physical examination, 12-lead ECG, 24-hour ambulatory ECG (Holter), exercise ECG, transthoracic echocardiography, magnetic resonance imaging of the heart, myocardial perfusion scintigraphy, and coronary angiography were collected. In cases of atrial fibrillation (AF) or pacemaker rhythm, earlier ECGs were analyzed for conduction disorders.

Pedigrees of the families were constructed to study segregation of the variant with the phenotype. Family members were considered to have cardiac involvement if there was evidence of sinus node dysfunction, supraventricular or ventricular arrhythmias, atrioventricular and ventricular conduction delay, pacemaker or ICD implantation, structural cardiac abnormalities (determined by echocardiography or magnetic resonance imaging), or symptomatic heart failure in the absence of other known causes. For a more detailed description of the phenotypes and definitions used, see Materials section in the Data Supplement.

Time-to-Event Analysis

Data of 56 carriers of a pathogenic *LMNA* mutation—not p.(Arg331Gln)—associated with DCM (31 probands and 25 family members) were used to compare outcome. Pathogenicities of these mutations were previously assessed using a clinical classification scheme described earlier.[13] Data of these *LMNA* mutation carriers have been analyzed and used in previous studies.[6,13,14] An event was defined as a composite of the following end points: appropriate ICD therapy, out-of-hospital cardiac arrest, heart transplant (HTx)/left ventricular assist device implantation, or death. Appropriate ICD therapy was defined as antitachycardia pacing or an ICD discharge for termination of ventricular tachycardia or fibrillation. A log-rank test was performed to evaluate the potential difference in outcome between the LMNA p.(Arg331Gln) mutation carriers and other *LMNA* mutation carriers, labeled LMNA group. We also compared the outcome of p.(Arg331Gln) carriers to those of individuals with only a missense *LMNA* mutation (16 probands and 11 family members) from the LMNA group, labeled LMNA missense only.

Haplotype Analysis and Genealogy

To evaluate whether the mutation originated from a common founder, 12 microsatellite markers around *LMNA* were analyzed. Verification of the phase and reconstruction of the haplotype was made possible by analyzing DNA samples of relatives. Calculation of the age of the haplotype was performed as described before with the assumption that a generation equals 20 years.[15] An estimation of the marker frequency in the general population of the first recombinant markers on both sides of the *LMNA* gene in the probands was made by analyzing these markers in 96 unrelated control individuals. Markers D1S305 and D1S2624 were used for this purpose.

To find common ancestors in these different families, we also performed genealogical searches using community registries and official records of births, marriages, and deaths.

Linkage Analysis

Linkage analysis was performed in the families D, E, G, I, L, M, P, and Q. For this purpose, we used the linkage program GRONLOD.[16] The model assumptions we used are described in the Materials section in the Data Supplement.

Nuclear Morphology of LMNA p.(Arg331Gln) Fibroblasts

For detailed information about the immunofluorescence staining, see Materials section in the Data Supplement. Fibroblasts obtained from a skin biopsy from a patient carrying the LMNA p.(Arg331Gln) variant were stained with the antibody JoL2 for detection of Lamin A/C and then counterstained using 4',6-Diamidino-2-Phenylindole. Structural abnormalities of the nuclei were scored based on abnormalities of nuclear shape and according to the following categories: normal, presence of herniations (blebs) and honeycomb structures, and presence of donut-like nuclear invaginations. They were also compared with nuclear morphology data available from control dermal fibroblast cultures.

Electronic Microscopic Imaging of the Nucleus

See Materials section in the Data Supplement for a detailed explanation of the electron microscopy (EM) imaging. Two myocardial biopsies of patients carrying the LMNA p.(Arg331Gln) mutation were fixed with Karnovsky Fixative, embedded in Epon, and cut into 70-nm sections. They were then viewed with a FEI Tecnai T12 Electron Microscope.

Maximal Force Development of the Sarcomeres

For a detailed description, Materials section in the Data Supplement. Single cardiomyocytes from patients carrying LMNA p.(Arg331Gln) and from control hearts were membrane permeabilized and glued between a force transducer and piezo motor. Force development was induced by transferring the cell to solutions of calcium with different concentrations (ranging from physiological concentrations to a saturating calcium concentration). Force development was recorded with the force transducer. In a later stage, maximal force generation was corrected for myofibril density, measured on EM images.

Statistical Analysis

Descriptive statistics are reported as frequency or mean±SD. We used Kaplan–Meier survival to determine the cumulative event-free survival in LMNA p.(Arg331Gln) carriers. We used the Log-rank test to compare the outcomes for LMNA p.(Arg331Gln) carriers to those of other pathogenic *LMNA* mutation carriers. For the Kaplan–Meier survival analyses, we used MedCalc Statistical Software version 17.1

(MedCalc Software bvba, Ostend, Belgium). An independent 2-sided *t* test was used to compare the nuclear irregularities. The data were analyzed with the Statistical Package for Social Sciences (SPSS software version 23.0; IBM Corp, Armonk, NY). Force development between groups was compared by Student *t* test after normal distribution was confirmed by Shapiro–Wilk normality test. Statistical analysis on force development was performed by GraphPad Prism 5 software. Data of the force measurements and myofibril density are shown as mean±SEM. A *P* value <0.05 was considered to represent a significant difference between groups.

Results

Mutation Analysis

The next-generation sequencing cardiomyopathy panel was performed in 22 probands. Fourteen additional variants were found in 13 probands with the targeted cardiomyopathy panel, of which one was labeled as pathogenic and the others as variant of unknown significance (Table I in the Data Supplement). Screening of the major lipodystrophy genes (*CAV1*, *PLIN1*, *PPARG*, and *AKT2*) with whole-exome sequencing was negative for the proband A-III. Analyses of cardiomyopathy-related genes screened with whole-exome sequencing identified no additional mutation (a list of the screened genes is available on request).

The LMNA p.(Arg331Gln) variant was found twice in the Exome Aggregation Consortium data set (allele frequency 0.0015%) and 4 times in the GoNL database (allele frequency 0.4%).[17,18]

Clinical Evaluation

For a complete overview of clinical features in mutation carriers, see Table and Table II in the Data Supplement. Twenty-three probands were identified, of whom 21 presented with cardiac symptoms, 1 with symptoms of a partial lipodystrophy, and 1 was identified after family screening after sudden cardiac death. Thirty-five family members were identified as carrying the mutation. Sixteen family members were already known to have cardiac symptoms before genetic family screening (8 men; mean age of presentation, 56±7 years), 18 family members were evaluated for the first time in the course of family screening (9 men; mean age at first clinical examination 47±12 years), and from 1 family member, no cardiological information was available. In both probands and family members, there was a high incidence of (paroxysmal) AF (52% and 42%, respectively) and atrioventricular conduction delay (61% and 44%, respectively). Ventricular arrhythmias were frequently reported in both groups, although the occurrence seemed to be higher in the proband group (83% versus 40%). Twenty two of 23 probands (96%) had structural abnormalities of the myocardium, of which 17 (74%) were classified as DCM. Structural abnormalities were present in only 11 family members (38%), of which 4 were classified as DCM. The overall mean age at the diagnosis of DCM was 50±15 years. End-stage heart failure was seen in 6 carriers, of whom 5 received an HTx. Two patients died of heart failure, of whom 1 received a left ventricular assist device implantation while awaiting a HTx. Two patients had an aborted cardiac arrest and 2 appropriate ICD therapy shocks were administered in total.

Explanted hearts of 2 patients after HTx showed extensive involvement of the right ventricle. The right ventricle even seemed to be predominantly involved in all 3 members of family Q. One family member fulfilled the revised Task Force

Table. Summary of Characteristics of the Probands and Family Members Carrying the LMNA p.(Arg331Gln) Mutation

Characteristics	Probands (n=23)	Family Members (n=35)
Age presentation/evaluation, y (n=23 and n=29)*	47±(14)	51±(12)
Male sex	17 (74)	18 (51)
Symptoms		
Palpitations (n=16 and n=20)*	8 (50)	6 (30)
Syncope (n=17 and n=22)*	4 (24)	2 (9)
NYHA class ≥3 (n=16 and n=28)*	5 (31)	2 (7)
AV block (n=18 and n=25)*		
First degree	8 (44)	10 (40)
Second degree	3 (17)	1 (4)
Intraventricular conduction delay (n=20 and n=28)*		
LBBB	11 (55)	5 (18)
RBBB	3 (15)	1 (4)
Aspecific	…	4 (14)
Supraventricular arrhythmias (n=23 and n=31)*		
Paroxysmal atrial tachycardia	4 (17)	3 (10)
(Paroxysmal) atrial fibrillation	12 (52)	13 (42)
Ventricular arrhythmias (n=23 and n=30)*		
>500 PVCs		2 (7)
NSVT	13 (57)	9 (30)
VT/VF	6 (26)	1 (3)
PM or ICD implantation (n=22 and n=34)*	17 (77)	6 (18)
Cardiomyopathy (n=23 and n=29)*		
DCM	17 (74)	4 (14)
Mild DCM	5 (22)	6 (21)
Other structural abnormalities	…	1 (3)
HTx or end-stage heart failure (n=23 and n=30)*	5 (22)	1 (3)
Comorbidity		
Hypertension (n=23 and n=29)*	4 (17)	8 (28)
Coronary artery disease (n=23 and n=28)*	1 (4)	3 (11)
Diabetes mellitus type 2 (n=23 and n=27)*	2 (9)	1 (4)
Dyslipidemia (n=23 and n=27)*	1 (4)	3 (11)
Medication (n=23 and n=26)*		
Antiarrhythmics	22 (96)	11 (42)
ACE inhibitor or ARB	22 (96)	6 (23)
Diuretics	14 (61)	7 (27)

Values are mean±(SD) or n (%). ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; AV, atrioventricular; DCM, dilated cardiomyopathy; HTx, heart transplantation; ICD, implantable cardioverter-defibrillator; LBBB, left bundle branch block; NSVT, nonsustained ventricular tachycardia; NYHA, New York Heart Association; PM, pacemaker; PVCs, premature ventricular beats; RBBB, right bundle branch block; and VT/VF, ventricular tachycardia/ventricular fibrillation.

*Number with available data (probands and family members). Data are summary of the data collected to last follow-up.

criteria for borderline arrhythmogenic right ventricular cardiomyopathy: he had 1 major criterion (regional akinesia and an end-diastolic volume >110 mL/m^2 measured by magnetic resonance imaging) and 1 minor criterion (nonsustained ventricular tachycardia observed during exercise ECG). In the other 2 family members, a widened right ventricle was observed (magnetic resonance imaging; end-diastolic volume of 103 and 109 mL/m^2) with a inhomogeneous contraction pattern. In one of them, there was also focal bulging of the right ventricle. The left ventricle function was normal in all 3 subjects.

In family A, the mutation seemed to segregate only with a partial lipodystrophy (Figure I in the Data Supplement). In both the proband (A-III) and her mother (A-II), there was loss of adipose tissue around the extremities and accumulation of adipose tissue on the abdomen, neck, and face. Other manifestations were hypertension and hypertriglyceridemia. The proband (A-III) in addition had acanthosis nigricans. The mother (A-II) additionally experienced hepatic steatosis and diabetes mellitus type 2. The maternal grandmother had diabetes type 2 and was told to have the same physical appearance as A-II and to have unspecified cardiac problems. She died suddenly aged 72 years. During follow-up, a dilated left ventricle (end-diastolic dimension 62 mm) with systolic dysfunction (ejection fraction of 46%) was observed in the proband (A-III; Figure I in the Data Supplement).

Time-to-Event Analysis

Thirteen LMNA p.(Arg331Gln) mutation carriers reached the composite end point: appropriate ICD therapy, resuscitation, HTx/left ventricular assist device implantation, or death. Median survival, that is, staying free of the composite end point, for the p.(Arg331Gln) group was 71 years (95% confidence interval, 58–84 years), which is in contrast to 57 years (95% confidence interval, 53–61 years) for carriers of other *LMNA* mutations. Compared with other *LMNA* mutation carriers (both grouped and carriers of only missense mutations), the composite event occurred significantly later in the LMNA p.(Arg331Gln) mutation carriers (log-rank $P<0.001$; Figure 1). Information on type of *LMNA* mutation and number of carriers is given in Table III in the Data Supplement. No significant differences were found regarding sex or proband status, comorbidity, or use of medication between the groups (Table IV in the Data Supplement).

Haplotype and Genealogy

Haplotype analysis was performed in 15 probands and 9 family members. A shared haplotype of at least 3 markers was found covering a 1.00 Mb region surrounding *LMNA* in all the 15 probands (Table IV in the Data Supplement). We calculated that the age of the haplotype containing the mutation is between 340 and 760 years old.

Through genealogical research, we found common ancestors in 6 families. We could genealogically link family A to family E 6 generations ago, family J to family M 6 generations ago (Figure I and Table IV in the Data Supplement), and family U to family S 4 generations ago (pedigrees not shown).

Linkage Analysis

A combined logarithm of the odds (LOD) score of 3.46 was found with linkage analysis in the families D, E, G, I, L, M, P, and Q (Materials section in the Data Supplement). This adds to the likelihood that LMNA p.(Arg331Gln) is linked to the disease. Functional analysis should further substantiate the hypothesis that the observed mutation is the causal one. In family F, the segregation was not conclusive. The cardiac phenotype of the LMNA p.(Arg331Gln)-negative mother, who had a reduced left ventricular ejection fraction (46%) and

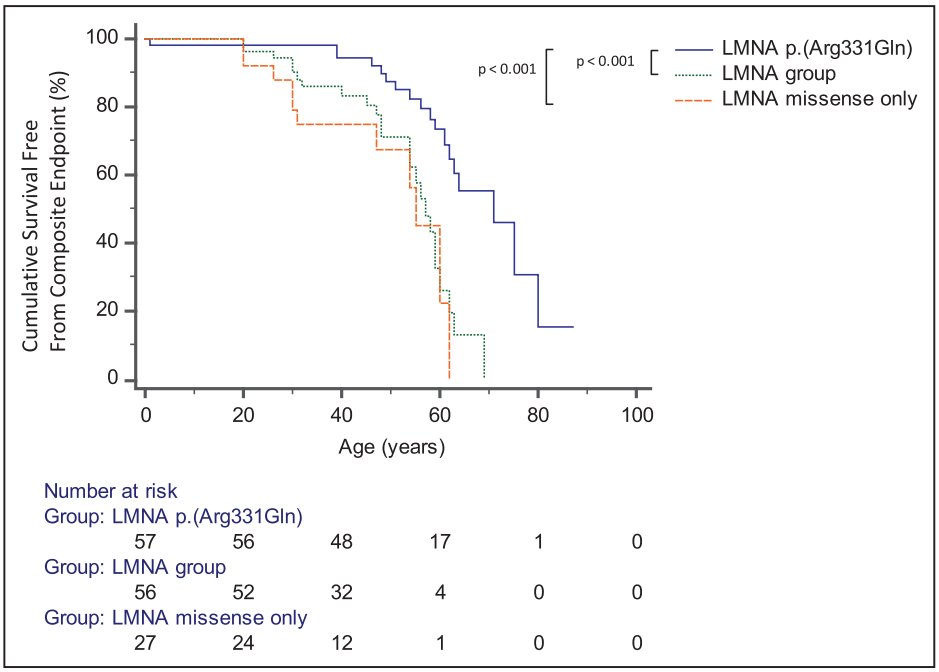

Figure 1. Kaplan–Meier survival analysis. Composite end point is appropriate implantable cardioverter-defibrillator treatment, resuscitation, heart transplant/left ventricular assist device, and death. LMNA p.(Arg331Gln) carriers had a significantly better outcome compared with the LMNA group, which composed of carriers of different types of *LMNA* mutations. The outcome was also compared with a subgroup of the LMNA group, which consisted of only *LMNA* missense mutation carriers, called the LMNA missense-only group.

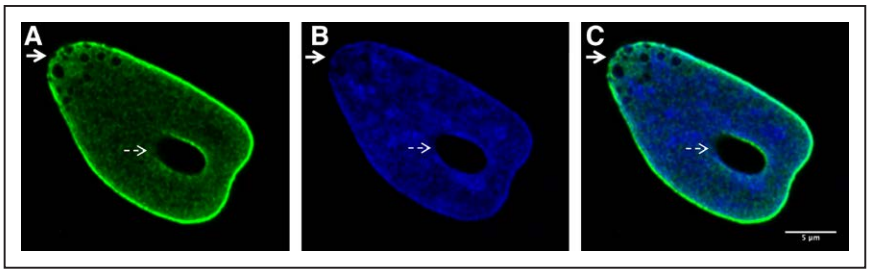

Figure 2. Nuclear envelope immunostaining of skin fibroblasts of an LMNA p.(Arg331Gln) carrier. **A**, Lamin A/C staining with antibody JoL2. **B**, 4',6-Diamidino-2-Phenylindole staining. **C**, Lamin A/C staining and 4',6-Diamidino-2-Phenylindole staining merged. Broken white arrow indicates donut-like nuclear invaginations. Continuous white arrow indicates honeycomb-like nuclear structure.

frequent ventricular ectopic beats (±18 000/24 h), could also be explained by the fact that she is a carrier of the pathogenic *SCN5A* p.(Gly1319Val) mutation (F-I-1, Figure I in the Data Supplement)[19] because there are more cases described in which *SCN5A* mutations are associated with DCM.[20,21] The family history reported that the father of the proband, an obligate carrier of the LMNA p.(Arg331Gln) mutation, died at the age of 56 years and that the paternal grandfather had a pacemaker.

Nuclear Morphology of LMNA p.(Arg331Gln) Fibroblasts

Nuclear morphology was analyzed using immunohistochemical staining for lamin A/C in fibroblasts of an LMNA p.(Arg331Gln) carrier (proband I-II-1). Next-generation cardiomyopathy panel analysis revealed no additional mutations in this patient. The morphology of 496 nuclei were analyzed. An irregular structure was observed in 22.0±6.4% of the p.(Arg331Gln) nuclei, with a honeycomb-like nuclear structure the most frequently observed irregularity (13.6±8.3%; Figure 2). Nuclear blebbing and donut-shaped nuclei were observed in 5.8±3.7% and 2.0±1.4% of the p.(Arg331Gln) fibroblasts, respectively. The findings are consistent with abnormalities of the nuclear membrane in other pathogenic *LMNA* mutations.[22] In contrast, the 8 control fibroblast cultures displayed fewer nuclear irregularities, 5.9±1.4% (P<0.01), of which 0.9±1.1% were honeycomb structures (P<0.01), 2.1±1.8% (P=0.04) were nuclear blebbing, and 1.4±0.7% (no significant difference) were donut-shaped nuclei (data not shown).

EM Imaging of the Nucleus

EM of myocardial biopsies of 2 patients demonstrated the irregular and convoluted shapes of the enlarged nuclei of the cardiomyocytes (Figure 3). In patient P-III-2 (family member), carrier of additional variant of unknown significance in the gene encoding desmoplakin (*DSP*) (DSP p.(Lys2706Met)), blebs of the nuclear membrane into the cytoplasm were observed (Figure 3B). In proband B, who carried 2 additional variants of unknown significance (LMNA p.(Arg156Leu) and TTN p.(Phe9717Serfs*23)), a discontinuous layer of heterochromatin of the inner nuclear membrane was observed in several areas of the nuclei. In this patient, some small indications of blebs were observed, but larger ones were not evident. Because Lamin A and C play a role in the structural stability of the nuclear membrane,

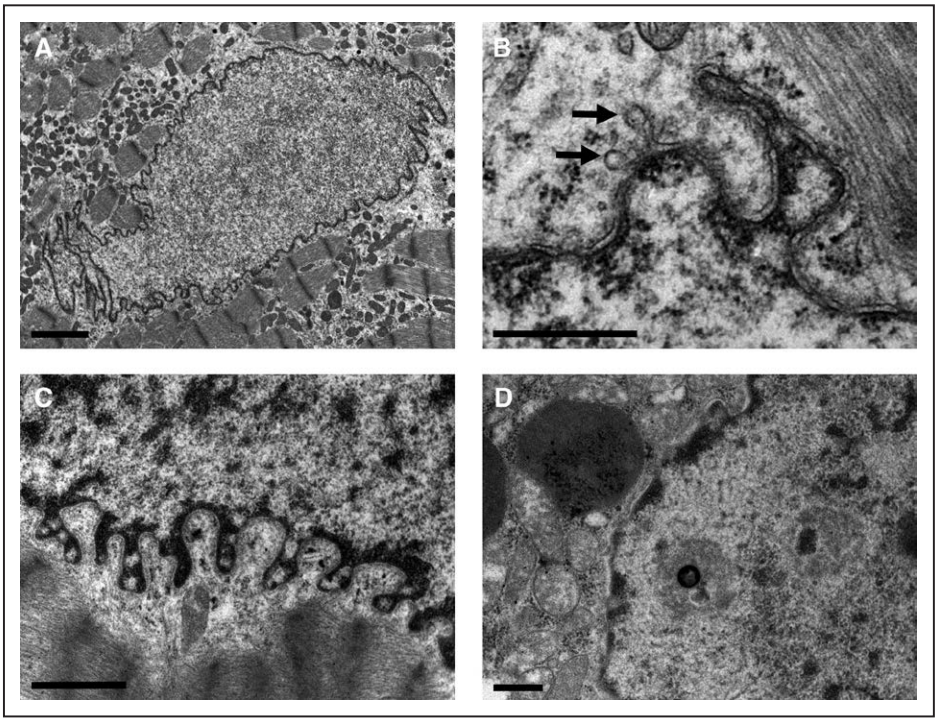

Figure 3. Electron microscopy of myocardium of patients P-III-2 and B-III-1. **A** and **B**, Patient from family P. **A**, Nucleus of cardiomyocyte with convoluted shape. Bar=2 μm. **B**, Detail of nuclear membrane with small blebs of the nuclear membrane into the cytoplasm (arrows). Bar=500 nm. **C** and **D**, Proband from family B. **C**, Irregular shape of the nuclear membrane. Bar=1 μm. **D**, Detail of nuclear membrane showing a discontinuous layer of chromatin of the nuclear membrane, possible enlarged nuclear pores. Bar=500 nm.

the ultrastructural defects of the nuclear membrane described above are often seen in conjunction with *LMNA* mutations.[23,24]

Maximal Force Development of the Sarcomeres

In Figure 4A, a cardiomyocyte of a control heart and in Figure 4B, a cardiomyocyte of a patient with the LMNA p.(Arg331Gln) variant is visualized at sarcomere length 2.2 µm. Cardiac tissue of 3 carriers (B-II-1, N, and P-III-2) was used for this analysis. B-II-1 carried 2 additional variants, and P-III-2 carried 1 additional variant of unknown significance (Table II in the Data Supplement). After transfer of the cell to a solution containing an activating concentration of calcium, the cell developed force, which was recorded by the force transducer. Patients with the LMNA p.(Arg331Gln) mutation showed a significantly decreased maximal force development (17.9 kN/m^2) compared with controls (28.5 kN/m^2; P=0.002; Figure 4C). This indicates an effect of the variant through impairment of cardiomyocyte contractility. As shown

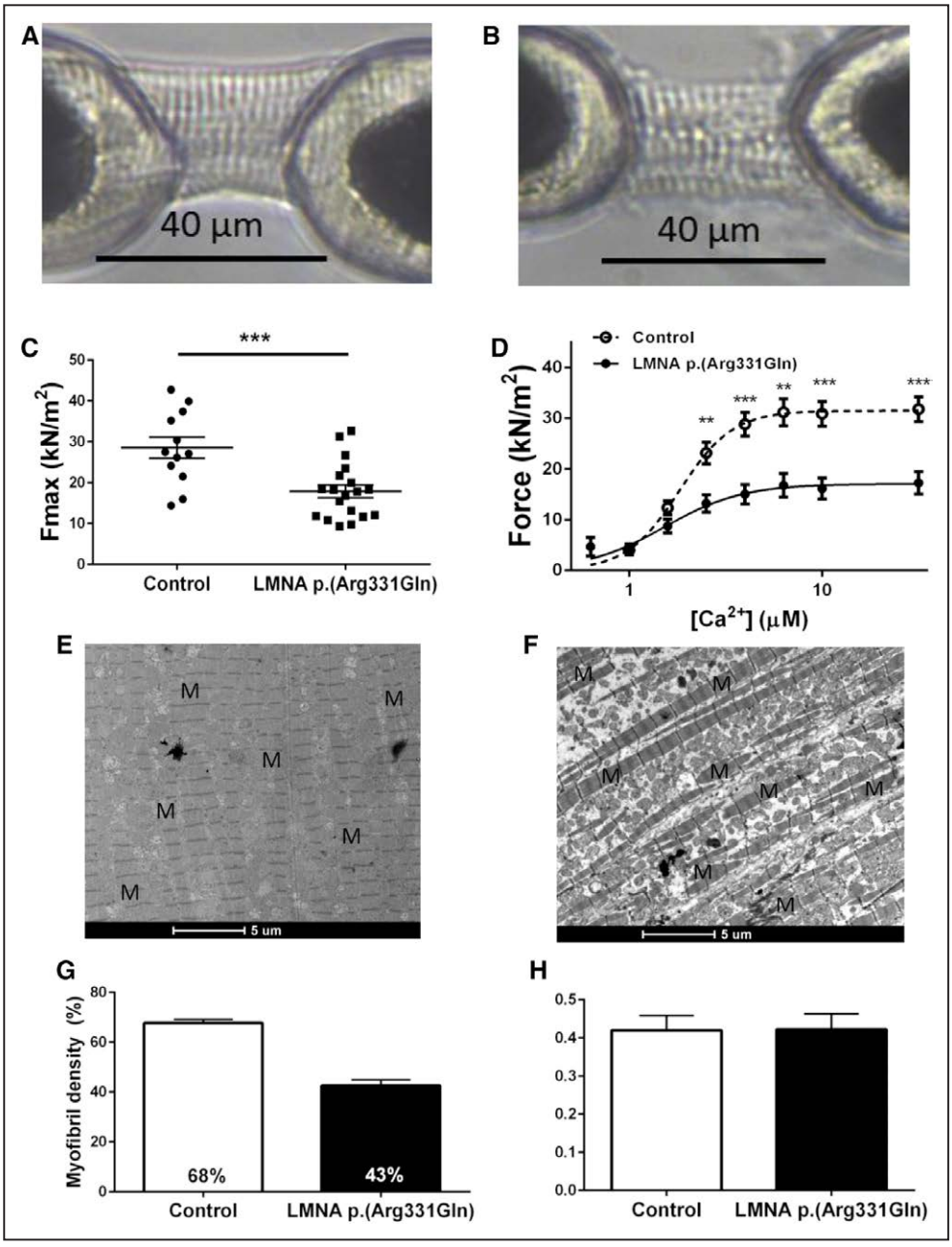

Figure 4. Maximal force development of the sarcomeres. **A** and **B**, Mechanical isolated cardiomyocyte of a control heart (**A**) and a cardiomyocyte of a patient with the LMNA p.(Arg331Gln) mutation (**B**) glued between a force transducer and piezo motor. **C**, Patients with the LMNA p.(Arg331Gln) mutation (N=3; n=19) showed a significantly decreased maximal force development compared with controls (N=2; n=12; P=0.002). **D**, Force development was significantly lower in p.(Arg331Gln) samples (N=3; n=11) compared with controls (N=2; n=9) over a range of submaximal (physiological) calcium concentrations. **E**–**G**, Myofibril density was lower (**F**) in the LMNA p.(Arg331Gln) patients (N=3; **G**)) compared with (**E**) control hearts (N=2; **G**). **H**, Maximal force development corrected for myofibril density was similar in the LMNA p.(Arg331Gln) patients (N=3; n=19) compared with the control hearts (N=2; n=12). For each sample, at least 4 electron microscopy images at a magnification of ×2250 were used to calculate average the myofibril density. N indicates number of patients; and n, number of cardiomyocytes.

in Figure 4D, force development was significantly lower in p.(Arg331Gln) variant samples compared with controls over a range of submaximal (physiological) calcium concentrations.

In some cases of hypertrophic cardiomyopathy, a reduction of myofibril density underlies the lower force-generating capacity.[25] We hypothesized that this could also be the case for the LMNA p.(Arg331Gln) variant. Myofibril density was calculated as a percentage of total cardiomyocyte area by EM, and myofibril density was found to be lower in the hearts of LMNA p.(Arg331Gln) patients (Figure 4F) when compared with control hearts (Figure 4E). Myofibril density was 43% in the LMNA p.(Arg331Gln) patient hearts compared with 68% in control hearts (Figure 4G). Maximal force development corrected for myofibril density was similar in the LMNA p.(Arg331Gln) patients compared with control hearts (Figure 4H). This indicates that the decreased force generation observed in the p.(Arg331Gln) mutation in LMNA is probably because of the reduced myofibril density.

Discussion

Interpretation of missense mutations is especially challenging when a variant is also present in a control population, the situation we encountered here for the LMNA p.(Arg331Gln)) variant. Although we are not the first to describe the LMNA p.(Arg331Gln) variant, we were able to collect the largest cohort of carriers to date. In a previous report, this variant was found in a patient who was compound heterozygous (carrier of the LMNA p.(Glu347Lys)). In addition, another variant at the same position, LMNA p.(Arg331Pro), was described to be associated with DCM, conduction delay, and limb-girdle muscular dystrophy.[26] In another report, the parents of the proband were not screened for the mutation and were seemingly unaffected (only the father had AF).[27] Extensive evaluation of clinical, segregation, and functional data helped us to classify this mutation as truly pathogenic, and we decided to communicate this as such to the carriers of this mutation. Moreover, its associated phenotype is consistent with that described in carriers of other *LMNA* mutations but milder in terms of significant clinical events (malignant arrhythmias, end-stage heart failure, or death).

The phenotype in our cohort is characterized by a high incidence of (paroxysmal) AF and atrioventricular conduction delay in both probands and family members ([paroxysmal] AF, 52% and 42% and atrioventricular block, 61% and 44%). These findings are consistent with the clinical findings of 2 studies of 269 and 299 *LMNA* mutation carriers.[4,6] These *LMNA* mutation carriers demonstrated a high prevalence of atrial tachyarrhythmia (36%) and conduction disease (47%).[6] More specifically, Fatkin et al[28] described 4 missense mutations in the rod domain, where the p.(Arg331Gln) variant is also situated, with a phenotype (atrioventricular conduction delay, AF, sinus bradycardia, and DCM) similar to that seen in our cohort. However, malignant ventricular arrhythmias (appropriate ICD therapy, sudden cardiac death, out-of-hospital cardiac arrest, and ventricular fibrillation) did not seem to occur as often (12%) in our cohort compared with *LMNA* patient series described in the literature, where malignant ventricular arrhythmias were observed in 24% to 28% of the cases.[6,29] The milder phenotype in our carriers is corroborated by the relatively infrequent occurrence of appropriate shock and antitachycardia pacing therapy (only 2 shocks and 3 antitachycardia pacing therapies in 86 patient-years). In both cases of ICD shock, there were additional factors that could have played a role (poor LV systolic function and evidence of an old myocardial infarction). This is in contrast to the observation in patients with other *LMNA* mutations where 28% to 42% of the carriers seemed to benefit (appropriate therapy) from ICD implantation.[6,8] The diagnosis of DCM in our cohort was made relatively late in life (50±15 years), compared with that of the group of DCM patients carrying other pathogenic *LMNA* mutations, for whom an age of onset of 40±10 years is described.[29] Structural abnormalities were only apparent in 11 family members (38%). However, 65% of the family members had electric disturbances of the heart (evidence of sinus node dysfunction, cardiac [atrioventricular] conduction delay, and atrial or ventricular arrhythmias, with no structural abnormalities of the heart [yet]). In *LMNA* mutations, it is a well-known phenomenon that electric abnormalities, like conduction delay and arrhythmias, often precede the structural abnormalities.[4,28] Regular follow-up is warranted because these initial electric abnormalities could be the first signs of structural abnormalities, which could be followed by an impaired function and LV dilatation.

One notable aspect of our study is the pleiotropic effect of the LMNA p.(Arg331Gln) mutation, which is demonstrated by the differences in phenotypes between the families. This is most striking in family A where the mutation seemed to segregate with a partial lipodystrophy phenotype. When partial lipodystrophy is associated with *LMNA* mutations, the disease is also referred to as FLDP2 (familial partial lipodystrophy type 2), which is an autosomal dominant disease that mostly results from missense mutations in the C-terminal region of the *LMNA* gene and is characterized by progressive abnormal subcutaneous adipose distribution.[30] However, mutations in the N-terminal head and α-helical rod domain in which the FLDP2 is accompanied by cardiomyopathy and conduction disorders have also been described.[31] The apparent absence of this lipodystrophic phenotype in the other families suggests that there is another possible genetic cause. However, screening of other major lipodystrophy genes was normal. Other results of the pleiotropic effects of this mutation are the findings of the apparent solely right ventricle involvement in family Q. Recently, another *LMNA* mutation (p.(Leu140_Ala146dup)) was described as associated with both arrhythmogenic right ventricular cardiomyopathy and DCM.[32] In 2 other studies, genetic screening in patients with arrhythmogenic right ventricle cardiomyopathy revealed 5 missense mutations and 1 nonsense mutation in *LMNA* in the absence of mutations in the desmosomal genes.[33,34] Although DCM was the predominant form of cardiomyopathy in our cohort, right ventricular involvement was seen in 57% of the patients with DCM, and the available pathology reports in 2 probands describe extensive right ventricle involvement. This suggests that *LMNA*-related disease may mimic arrhythmogenic right ventricular cardiomyopathy. The heterogenous phenotype might be influenced by additional genetic factors (Table II in the Data Supplement), yet this series of patients is too small to systematically evaluate this.

Marker analysis showed a common haplotype of 1 Mb, suggesting a founder mutation. Slippage during replication of DNA in one of the ancestors could explain the difference in length of marker D1S1153 found in the 2 groups.

Although most of the families were small, a dominant autosomal inheritance pattern could be observed. The calculated combined LOD score was well >3, an additional observation suggesting pathogenicity of this variant. A limitation of the segregation analysis is the fact that we counted a subject as affected when he or she displayed one of the phenotypes commonly observed with *LMNA* mutations. As described earlier, the phenotype can be highly variable, and some of the phenotypes, for example, AF and conduction disease, are also found in relatively high frequencies in the general population in absence of *LMNA* mutations. To take this into account, we calculated the LOD scores with a phenocopy frequency (eg, AF 10% and conduction disease 10%) higher than expected for the general population (Materials section in the Data Supplement). Still, that resulted in a LOD score of >3. Nonsegregation was observed possibly once and has been described before in a large *LMNA* family.[35] In family F, the cardiac phenotype of the mother (F-I-1) could be explained by a pathogenic *SCN5A* mutation, as it is recognized that *SCNA5A* mutations can also cause DCM.[20] The same *SCN5A* mutation was also found in our laboratory in 2 unrelated patients with cardiomyopathy, whereas screening of 53 or 55 other cardiomyopathy-related genes revealed no additional mutations in those subjects (unpublished data, Hoorntje 2017).

Lamins A and C are important components of the nuclear envelope and, when mutated, abnormalities of the nuclear envelope can be observed.[22] Irregular nuclear structures were significantly more frequently observed in fibroblasts of a LMNA p.(Arg331Gln) carrier. The nuclear abnormalities observed in the p.(Arg331Gln) fibroblasts are in line with the nuclear irregularities observed in fibroblasts of other established pathogenic mutations in *LMNA*, thereby supporting its pathogenicity.[22,36–38]

Like the abnormal nuclear structures in the fibroblasts observed using immunofluorescence, ultrastructural investigation on diseased cells in cardiac tissue with EM also support pathogenicity. The convoluted shapes of the nuclei, blebs, discontinuous layer of heterochromatin, and possible enlarged nuclear pores are features commonly seen in other *LMNA* mutations.[23,24,39] It should be kept in mind that such structural defects can also be found in DCM patients without *LMNA* mutations.[40]

Apart from their function in nuclear stability, it has been suggested that lamin proteins are important for the structural integrity of the whole cell through interactions between nuclear lamina, the cytoskeleton, and the extracellular matrix.[41,42] The lamin A/C coil 2B domain in which the p.(Arg331Gln) mutation is located is important for homodimerization of lamin proteins. Gangemi et al[43] indicated that the p.(Arg331Gln) mutation might affect lamina stability, because it has been predicted to impair dimerization of the lamin proteins because of loss of salt-bridge interactions. This might explain the detrimental effect on the heart because correct assembly of dimers is essential for protein function. In addition, it is known that myofilaments in cardiomyocytes create nuclear deformation in the plane parallel to the myofilaments during contraction.[42] Therefore, the continuous mechanical stress during contractions in cardiomyocytes can have pathological effects on nuclear structure over time in patients with the p.(Arg331Gln) mutation. Our study supports this possibility by showing impairment of nuclear architecture and decreased myofibril density in patients with the p.(Arg331Gln) mutation causing a reduction in cardiomyocyte force development.

Limitations

The observational design of this study is prone to introduction of survival bias. Initially, genetic testing focused on the most severely affected cases and currently patients with a less severe phenotype are more easily referred for genetic testing. However, this study includes all patients who were identified since the start of *LMNA* screening in 2001 and therefore is likely to reflect both sides of the spectrum. The combined LOD score of >3 does not mean significant linkage because of a possible selection bias in the families selected. It should be considered a strong indication that the mutation segregates with the observed phenotypes.

Conclusions

Genetic and segregation data support the pathogenic effects of LMNA p.(Arg331Gln). Electron microscopy and immunofluorescence showed an effect on nuclear architecture. In addition, the LMNA p.(Arg331Gln) mutation causes decreased myofibril density resulting in reduced force development at saturating and physiological calcium concentrations. The clinical phenotype related to the LMNA p.(Arg331Gln) founder mutation is generally characterized by a phenotype (consisting of cardiac conduction delay, (atrial) arrhythmias, and dilated cardiomyopathy with a later onset and more favorable prognosis compared with other pathogenic *LMNA* mutations. Further research is needed to elucidate the role of other contributing factors leading to the clinical variability.

Appendix

From the Department of Genetics (E.T.H., G.J.t.M., A.J.v.E., P.C.v.d.A., J.D.J., J.P.v.T.) and Department of Cardiology (M.P.v.d.B.), University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; Netherlands Heart Institute, Utrecht, The Netherlands (E.T.H., J.v.d.V., J.P.v.T.); Department of Physiology, Amsterdam Cardiovascular Sciences, VU Medical Center, Amsterdam, The Netherlands (I.A.B., J.v.d.V.); Department of Clinical Genetics (D.Q.B.-S., E.A.) and Department of Cardiology (M.B.), University of Leiden, Leiden University Medical Center, Leiden, The Netherlands; Unit Clinical Genomics, Department of Clinical Genetics (F.H.v.T., A.v.d.W.), Department of Cardiology (P.G.V.), and Department of Molecular Cell Biology, CARIM-School for Cardiovascular Diseases (J.L.B.), University of Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands; Heart Centre, Department of Clinical and Experimental Cardiology (J.A.J., A.A.W.) and Department of Clinical Genetics (K.Y.v.S.-Z., J.P.v.T.), Amsterdam Cardiovascular Sciences, University of Amsterdam, Academic Medical Center Amsterdam, Amsterdam, The Netherlands; Department of Cardiology (A.A.C.) and Department of Genetics (R.A.O.), University of Rotterdam, Erasmus Medical Center, Rotterdam, The Netherlands; Department of Genetics, University of Nijmegen, Radboud University Medical Center, Nijmegen, The Netherlands (C.L.M.); and Department of Genetics (J.J.v.d.S., E.A.H.) and Department of Pathology (A.V.), University of Utrecht, University Medical Center Utrecht, Utrecht, The Netherlands.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Dilated cardiomyopathy is characterized by an impaired systolic dysfunction and dilatation of the left ventricle. Although genetically heterozygous, *LMNA* is one of the most frequent genes found to be mutated in dilated cardiomyopathy. *LMNA*-related cardiac disease is associated with a high incidence of malignant ventricular arrhythmias and patients with an *LMNA* mutation are believed to have a poor prognosis. With the increasing use of next-generation sequencing based on dedicated gene panels, variants leading to an abnormal protein will be identified more often. Correct interpretation of these genetic variants is, however, challenging, especially when the variant is also found in control populations, as is the case for the LMNA p.(Arg331Gln) variation. After evaluating the clinical data, performing segregation analysis, and studying the nuclear morphology in cardiomyocytes and fibroblasts, we have classified this variant was as pathogenic. This allows for detection of asymptomatic mutation carriers and monitor them for early signs of disease and early intervention. Additionally, we show that LMNA p.(Arg331Gln) carriers seem to have a more favorable clinical course compared with patients with other *LMNA* mutations. This provides clinicians with the opportunity to address uncertainties regarding the disease course. Our data also provide novel insight into the possible pathogenesis of dilated cardiomyopathy caused by *LMNA* mutations, by showing that the decreased force development in the cardiomyocytes is likely to be because of secondary disease remodeling.