European Heart Journal (2012) **33**, 1128–1136
doi:10.1093/eurheartj/ehr451



# Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy

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*Received 29 July 2011; revised 25 October 2011; accepted 16 November 2011; online publish-ahead-of-print 23 December 2011*

**Aims** Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart muscle disease predominantly caused by mutations in desmosomal protein genes. Lamin A/C gene (*LMNA*) mutations are associated with dilated cardiomyopathy, conduction abnormalities and high incidence of sudden cardiac death. In this study, we screened a large cohort of ARVC patients for *LMNA* mutations.

**Methods and results** One hundred and eight patients from unrelated families with borderline ($n = 27$) or definite ($n = 81$) diagnosis of ARVC were genetically tested for five desmosomal genes and *LMNA*. Sixty-one (56.5%) were positive for desmosomal gene mutations. Standard polymerase chain reaction (PCR) amplification of the 12 protein-coding *LMNA* exons was performed and mutational screening performed by direct sequencing. Four patients (4%) without desmosomal gene mutations carried *LMNA* variants. Three had severe right ventricular involvement, and during follow-up three died (two suddenly and one from congestive heart failure); all three had conduction abnormalities on resting 12-lead electrocardiogram (ECG). Myocardial tissue from two patients showed myocyte loss and fibro-fatty replacement. In one of these, immunohistochemical staining with antibody to plakoglobin showed reduced/absent staining of the intercalated discs in the myocardium.

**Conclusion** Lamin A/C gene mutations can be found in severe forms of ARVC. Lamin A/C gene should be added to desmosomal genes when genetically testing patients with suspected ARVC, particularly when they also have ECG evidence for conduction disease.

**Keywords** Arrhythmogenic right ventricular cardiomyopathy • Lamin A/C gene mutations

## Introduction

Lamin A/C gene (*LMNA*) mutations cause a diverse range of clinical phenotypes including muscular dystrophy,[1,2] peripheral neuropathy,[3] lipodystrophies,[4–6] and premature ageing syndromes.[7–9] In the heart, *LMNA* mutations classically manifest as dilated cardiomyopathy associated with atrial arrhythmia, atrioventricular block, progressive heart failure, and a high incidence of sudden cardiac death.[10] Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart muscle disease characterized clinically by ventricular arrhythmias of right ventricular (RV) origin[11] and histologically by myocyte death and fibro-fatty replacement. Left ventricular (LV) involvement can occur with disease progression and in some cases is the predominant abnormality.[12]

Lamin A/C is a nuclear envelope protein involved in numerous signalling pathways including adipogenesis. Mutations in the *LMNA* cause a diverse range of phenotypes including dilated cardiomyopathy associated with a high incidence of ventricular arrhythmia. In this study, we hypothesized that mutations in *LMNA* may also cause ARVC.

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# Methods

The Heart Hospital, UCLH, London, UK is a national referral centre for inherited heart muscle diseases. One hundred and eight patients from unrelated families with borderline ($n = 27$) or definite ($n = 81$) diagnosis of ARVC according to the 2010 ARVC Criteria[11] were genetically tested for mutations in five desmosomal genes (plakoglobin, desmoplakin, plakophilin-2, desmoglein-2, desmocollin-2[13–16]) and *LMNA*.

All patients were evaluated in a dedicated ARVC clinic using clinical history, 12-lead electrocardiogram (ECG), signal averaged electrocardiogram (SAECG), transthoracic echocardiography, symptom limited upright bicycle exercise testing, 24-h ECG and, when necessary, contrast echocardiography and/or magnetic resonance imaging and endomyocardial biopsy.

## Genetic analysis

Genomic DNA was extracted from peripheral blood samples using a DNA extraction kit supplied by Qiagen. Mutational screening of the desmosomal genes involved in ARVC has been previously described.[13–15] Standard polymerase chain reaction (PCR) amplification of the 12 protein-coding *LMNA* exons (Ensembl transcript no. ENST00000368300) was carried out using intronic primers flanking each exon as previously described.[17,18] Polymerase chain reaction conditions are available on request. Mutational screening of PCR amplicons was performed by direct sequencing on an ABI 3130xl genetic analyser (Applied Biosystems) using BigDye Terminator chemistry (v3.1) according to standard protocols. Each fragment was sequenced in sense and anti-sense directions and identified sequence variants were confirmed in a duplicate DNA sample. Analysis of sequencing data was carried out using Seqscape v2.5 software (Applied Biosystems).

The UMD-*LMNA* mutations database was used to identify previously reported *LMNA* sequence variants.[19] The likelihood of a pathogenic effect of novel *LMNA* sequence variants was determined by three *in silico* prediction methods: The Grantham score,[20] PolyPhen-2,[21] and SIFT.[22]

DNA samples from 300 randomly selected, unrelated UK Caucasian blood donors (HRC DNA panels, European Collection of Cell Cultures) served as controls for each *LMNA* sequence variant identified in this study.

## Histology

A dissected, formalin-fixed heart was available for gross and histological examination in one patient who died suddenly. Immunohistochemical staining with antibody to plakoglobin was performed as previously described.[23] An endomyocardial biopsy was available for histological examination in one other patient.

## Ethical approval

The study was approved by the local research Ethics Committee. All patients gave informed written consent for genetic analysis. This study conforms to the ethical principles of Declaration of Helsinki on human research.

# Results

A total of 73 desmosomal gene mutations were found in sixty-one (56.5%) patients with the following distribution: desmoplakin, $n = 17$ (23.3%); plakophilin-2, $n = 25$ (34.2%); desmocollin-2, $n = 9$ (12.3%); and desmoglein-2, $n = 22$ (30.1%). Lamin A/C gene mutations were identified in four patients, none of whom had desmosomal gene mutations. Their pedigrees are shown in *Figure 1* and their clinical characteristics are summarized in *Table 1*.

## Family A

Patient A presented at the age of 47 years with syncope while walking. Her father died suddenly at the age of 56 years, but no further information was available. On admission to hospital, her resting 12-lead ECG showed sinus rhythm with complete right bundle branch block (RBBB). An echocardiogram showed mild biventricular dilatation and mild biventricular systolic dysfunction. She had episodes of non-sustained ventricular tachycardia (NSVT) with left bundle branch block (LBBB) morphology and inferior axis (*Figure 2A*) and during an exercise test developed sustained ventricular tachycardia. Coronary angiography demonstrated normal arteries. She was started on beta-blockers. During follow-up, she developed first-degree AV block and T-wave inversion in V1–V4 (*Figure 2B*). A cardiac magnetic resonance scan showed severe RV enlargement, with diffuse regional wall motion abnormalities and thinning, severe RV systolic dysfunction, and moderate LV systolic dysfunction. There was late gadolinium enhancement on the RV side of the basal septum with no evidence of fatty infiltration. An endomyocardial biopsy demonstrated >50% loss of myocytes, increased interstitial fibrous tissue, and accumulation of adipocytes (*Figure 2C* and Supplementary material online, *Figure S1*). She died suddenly aged 54 years, but no autopsy was available. *Post-mortem*, she was found to carry a c.568C > T (p.Arg190Trp) mutation in exon 3 of the *LMNA*. One of her sons aged 25 years is a carrier of the same mutation and has normal ECG and echocardiogram; a short run of NSVT has been detected during a 24-h ECG recording.

## Family B

Patient B presented at the age of 67 years with chest pain and palpitation. There was a family history of ischaemic heart disease and aortic aneurysm, but not of sudden cardiac death. Subsequent investigations showed angiographically normal coronary arteries, an abnormal ECG with flat T waves in V1–V4, a positive signal averaged ECG, and mild RV dilatation and mild RV systolic dysfunction with regional wall motion abnormalities in the RV free wall on echocardiography. Two hundred ventricular ectopics were recorded on 24-h ECG monitoring. She was found to carry a c.1930C > T (p.Arg644Cys) missense change in exon 11 of the *LMNA*.

## Family C

The third patient presented in 2001 aged 40 years with congestive heart failure but was referred for evaluation in 2008. Family history was unremarkable. ECG showed atrial fibrillation, complete right bundle branch block and T-wave inversion V1–V5. Echocardiography showed mild LV systolic dysfunction, a severely dilated right ventricle and severe RV systolic dysfunction. The patient deteriorated rapidly and died at the age of 48 years from congestive heart failure. No autopsy data were available. *Post-mortem*, she was found to carry a novel c.214C > T (p.Arg72Cys) missense change in exon 1 of the *LMNA*.

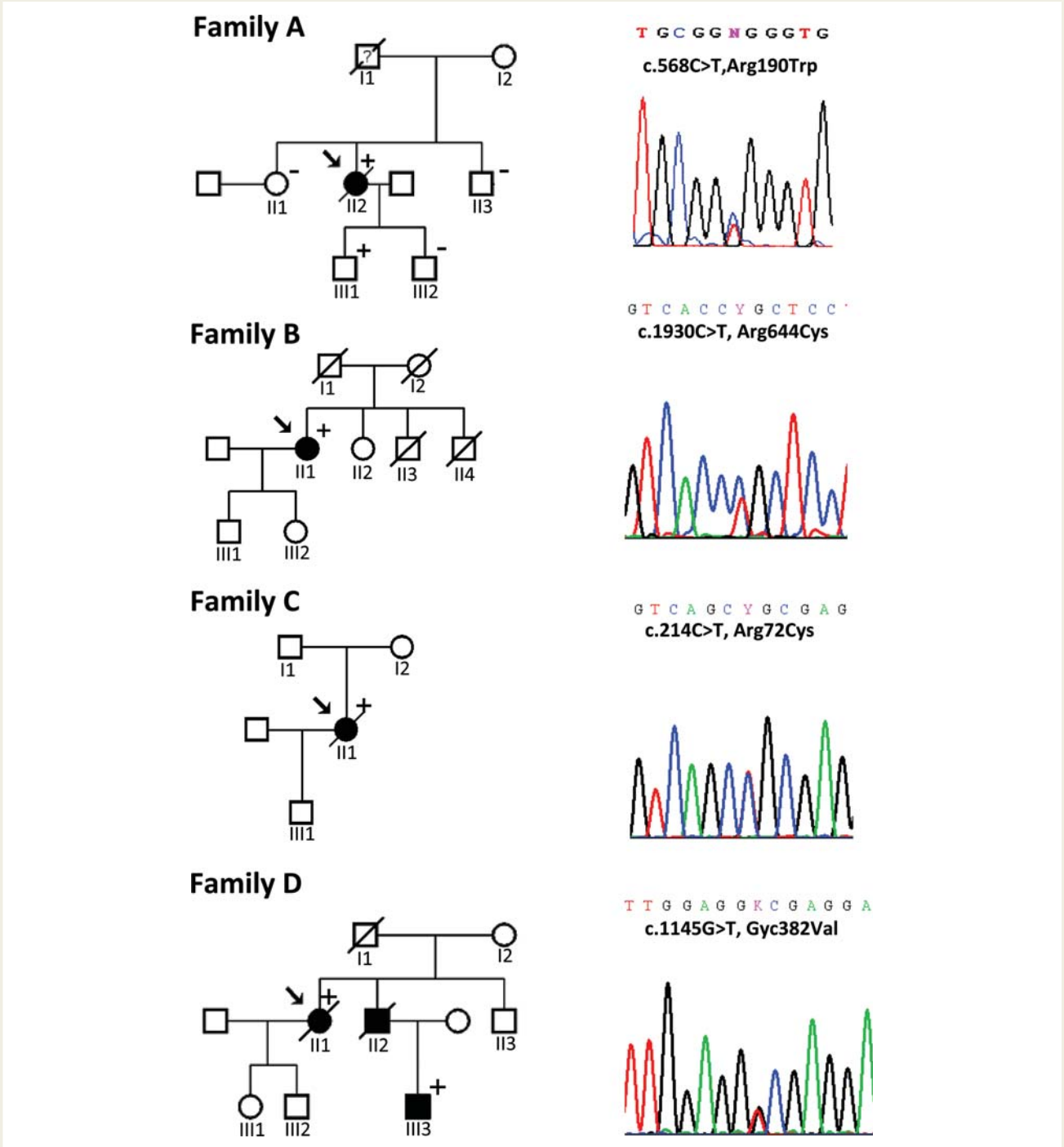


**Figure 1** Family pedigrees of the patients with a Lamin A/C gene variant.

## Family D

The fourth patient was diagnosed with atrial fibrillation during a pre-operative assessment at the age of 61 years. She underwent permanent pacemaker implantation two years later after a 24 h ECG recording showed a 3.5 s pause. An echocardiogram at this time demonstrated mild LV systolic impairment. Over a 3-year period, she developed progressive RV dilatation and systolic dysfunction. Her unpaced ECG showed sinus rhythm, first-degree AV block, small QRS complexes in the limb leads, poor R-wave progression, and T-wave inversion from V1 to V5 and inferior leads and flat T-waves in V6 and I and aVL (*Figure 3*). Coronary angiography was normal. She was commenced on anti-failure therapy but died suddenly at the age of 67 years. A post-mortem showed a dilated RV with >50% loss of myocytes, extensive interstitial fibrosis, fatty replacement, and no inflammatory cell infiltration (*Figure 4A–C* and Supplementary

**Table 1** Clinical characteristics of the arrhythmogenic right ventricular cardiomyopathy families with Lamin A/C gene variants

| ID | *LMNA* | Fh ARVC | Structural abnormalities | TWI in precordial leads | SAECG | Ventricular arrhythmias | Histology | Major/minor criteria | Diagnosis |
|---|---|---|---|---|---|---|---|---|---|
| Family A | | | | | | | | | |
| II 2 | c.568C > T (p.Arg190Trp) | — | Severe RV dilatation and systolic dysfunction with RWMA | RBBB TWI V1–V4 | NA | Sust VT | >50% myocytes loss, interstitial fibrosis and fatty replacement | 2/2 | Definite |
| III 1 | c.568C > T (p.Arg190Trp) | Fh ARVC in first-degree relative | — | — | — | NSVT | NA | 1/1 | Borderline |
| Family B | | | | | | | | | |
| II 1 | c.1930C > T (p.Arg644Cys) | — | Minor RV dilatation and systolic dysfunction with RWMA | TWI V1–V4 | + | ≈200 PVCs/24 h | NA | 1/2 | Definite |
| Family C | | | | | | | | | |
| II 1 | c.214C > T (p.Arg72Cys) | — | Severe RV dilatation and systolic dysfunction Mild LV systolic dysfunction | RBBB TWI V1–V5 | NA | — | NA | 1/1 | Borderline |
| Family D | | | | | | | | | |
| II 1 | c.1145G > T (p.Gly382Val) | — | Severe RV dilatation and systolic dysfunction Mild LV systolic dysfunction | TWI V1–V5 | NA | — | >50% myocytes loss, extensive interstitial fibrosis and fatty replacement | 3/0 | Definite |
| III 3 | c.1145G > T (p.Gly382Val) | Fh ARVC in second-degree relative | — | TWI V1–V3, flat T-waves V5–V6 | NA | — | NA | 1/1 | Borderline |

*LMNA*, Lamin A/C gene; Fh ARVC, Family history of arrhythmogenic right ventricular cardiomyopathy; RV, right ventricular; RWMA, regional wall motion abnormalities; RBBB, right bundle brunch block; TWI, T-wave inversion; SAECG, signal average ECG; PVCs, premature ventricular complexes; Sust VT, sustained ventricular tachycardia; NSVT, non-sustained ventricular tachycardia; NA, not available/applicable; a plus sign means present; a minus signs means absent.

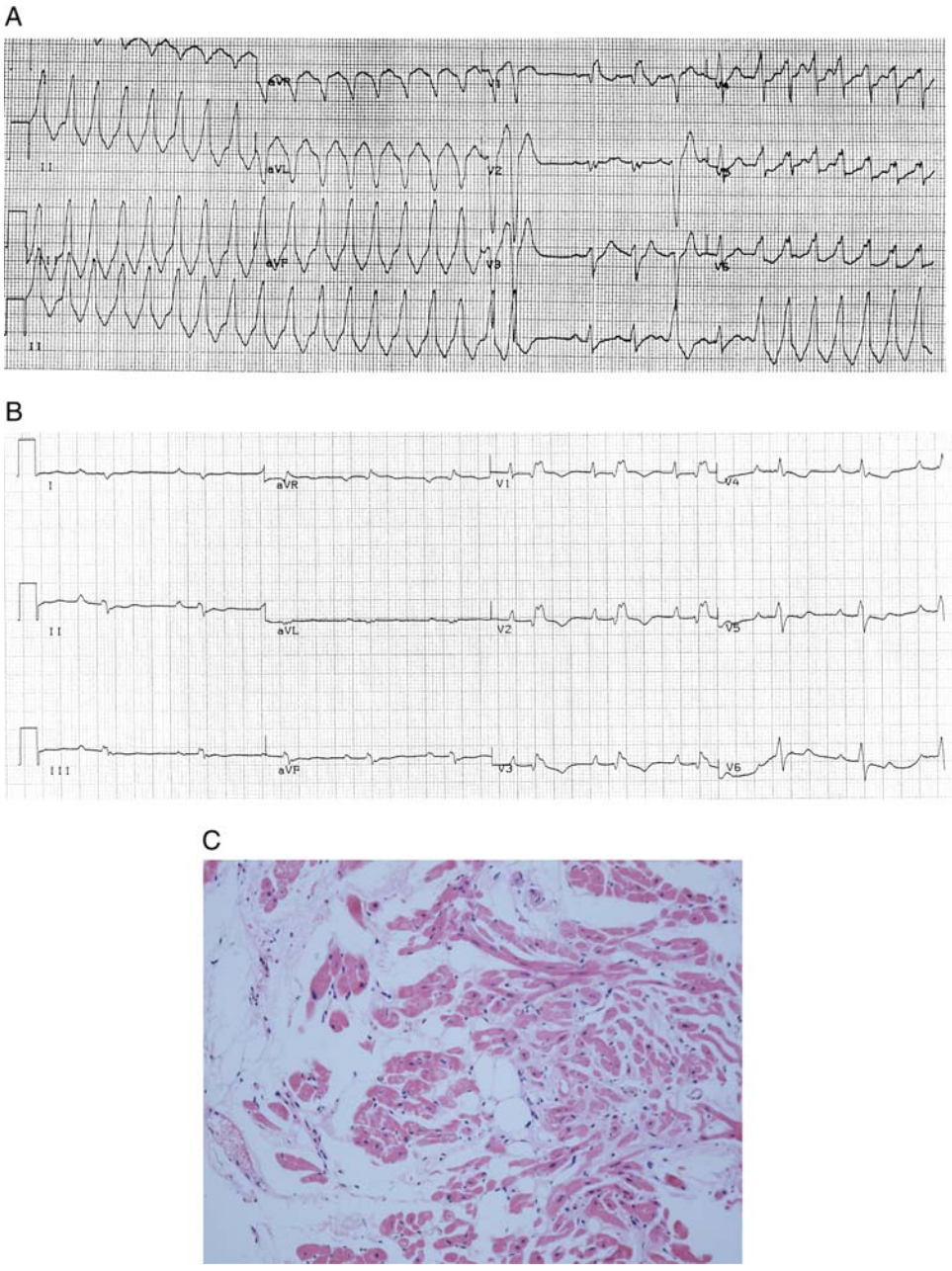


**Figure 2** Clinical and histological characteristics of patient A. (*A*) Episodes of non-sustained ventricular tachycardia with left bundle branch block morphology and inferior axis; (*B*) electrocardiogram showing sinus rhythm, atrial dilatation, first-degree atrio-ventricular block, complete right bundle branch block, and T-wave inversion V1–V4 and small QRS complexes in limb leads; (*C*) biopsy of right ventricular myocardium. There are irregular and enlarged myocyte nuclei, in keeping with myocyte hypertrophy. There is an increase in interstitial fibrous tissue and accumulation of adipocytes in the fibrotic areas. The appearances are in keeping with arrhythmogenic cardiomyopathy (haematoxylin and eosin staining, original magnification ×40).

material online, *Figure S2A* and *B*). There was focal fibrosis in the left lateral wall with no associated fatty replacement. His-bundle section showed mild fibrosis (Supplementary material online, *Figure S3*). Immunohistochemical staining for plakoglobin showed greatly reduced/absent staining at the intercalated disc (*Figure 4D–E*). Genetic testing *post-mortem* revealed a novel missense c.1145G > T (p.Gly382Val) *LMNA* variant. Her brother had dilated cardiomyopathy and died suddenly in 1987 aged 58 years; his son was diagnosed with paroxysmal atrial fibrillation with episodes of bradycardia and a permanent pacemaker was implanted. His unpaced ECG showed sinus bradycardia, poor R-wave progression and T-wave inversion in V1–V3, and flat T-wave in V4–V5 (*Figure 5*). His echocardiogram was normal. He carries the same *LMNA* variant as his aunt.

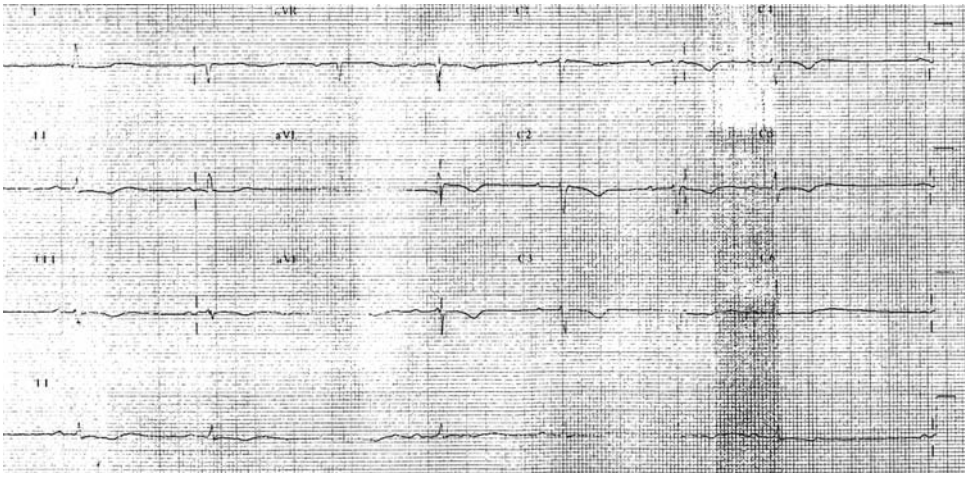

**Figure 3** Clinical characteristics of patient D. Electrocardiogram showing sinus rhythm, poor R-wave progression, T-wave inversion V1–V5 and in inferior leads, and small QRS complexes in limb leads.

## Pathogenicity of individual mutations

Arg190Trp affects a conserved amino acid position (Supplementary material online, *Table S1*) in the central rod domain of Lamin A/C which interacts with emerin and lamin B.[24] It is reported in patients with dilated cardiomyopathy with atrioventricular block[25] and more recently in a family with dilated cardiomyopathy and LV non-compaction.[26]

Arg644Cys is located in the C-terminal globular domain of lamin, which is responsible for interactions with chromatin and various proteins including emerin, nesprin, and actin.[24] It is located in the prelamin A at the level of a cleavage site of the endoprotease ZMPSTE24, an essential step in the formation of the mature Lamin A/C. The arginine residue at position 644 of *LMNA* is highly conserved across several species (Supplementary material online, *Table S1*). Clinically, the Arg644Cys variant is associated with a more diverse phenotype including dilated cardiomyopathy,[27,28] dilated cardiomyopathy and left-ventricular non-compaction,[29] hypertrophic cardiomyopathy,[28] lipodystrophy, peripheral neuropathy, Emery Dreifuss and limb girdle muscular dystrophies,[28–30] and atypical progeria.[31]

The novel c.214C > T missense change is located in exon 1 of *LMNA* and leads to the substitution of arginine with cysteine at amino acid position 72 (p.Arg72Cys). Arg72 is a highly conserved position (Supplementary material online, *Table S1*) in the central rod domain located between coil 1a and 1b. Two mutations close to position 72 have been reported in patients with Emery Dreifuss muscular dystrophy (EDMD) (c.194A > G, p.Glu65Gly,[32] and c.203_208del, p.Glu68_Val69del[33]) and with limb girdle muscular dystrophy 1B (c.194A > G, p.Glu65Gly[32]).

The novel missense mutation (c.1145G > T, p.Gly382Val) in exon 6 affects a conserved amino acid position (Supplementary material online, *Table S1*) in the central rod domain of lamin. A mutation in the same position resulting in a different amino acid substitution (c.1144G > C, Gly382Arg) is reported in a patient with EDMD on the Universal Mutation Database.[18] The variant in this study co-segregated with disease in the family.

None of the above LMNA sequence changes was detected in 300 control samples indicating that they are not commonly occurring variants. In addition, we have assessed the likelihood of pathogenic effect of LMNA sequence variants identified in this study by using three *in silico* prediction methods: The Grantham score[20] PolyPhen-2,[21] and SIFT.[22] All four mutated variants were predicted to affect the lamin protein structure, which provides further evidence that these variants are associated with disease in our families (Supplementary material online, *Table S2*).

## Discussion

To the best of our knowledge, this is the first report of ARVC caused by mutations in *LMNA*. The clinical phenotype was identical to that reported in ARVC patients in association with desmosomal gene mutations except for the presence of atrial arrhythmias and conduction system disease. The histological appearances in two individuals were identical to those of classical ARVC.

Systematic family studies have shown that ARVC is inherited in up to 50% of cases.[34] While the mode of transmission in the majority of patients is autosomal dominant with incomplete penetrance and variable clinical expression, the first disease causing mutation was identified in an autosomal recessive syndromic form of ARVC (Naxos disease)[35] caused by a homozygous base pair deletion in the gene encoding plakoglobin, a member of the armadillo protein family found in adherens and desmosomal intercellular junctions. Mutations in another desmosomal protein, desmoplakin,[36] were subsequently identified in similar autosomal recessive syndromic forms of ARVC in South American and Arab families. Since then, heterozygous mutations in genes encoding these and other desmosomal proteins have been identified in up to 70% of patients with non-syndromic autosomal dominant forms of ARVC.[37]

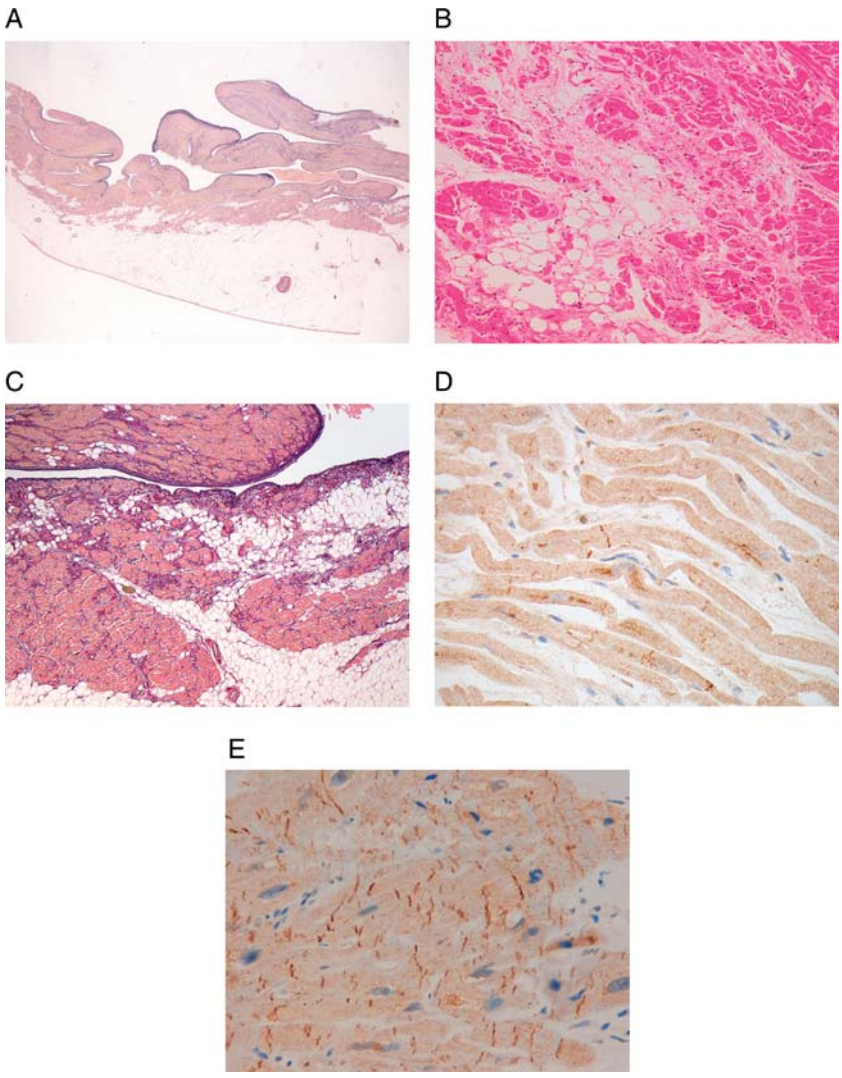


**Figure 4** Histological characteristics of patient D. (*A*–*C*) A section from the right ventricular myocardium (images a and c: Elastic van Gieson staining; image b: haematoxylin and eosin staining). There is loss of myocytes with replacement fibrosis and fatty infiltration. This change was extensive in the right ventricular wall; (*D*) A section from the right ventricular myocardium stained with antibody to plakoglobin. The intercalated discs stain as sharply defined dark brown lines perpendicular to the long axis of the myofibre. There is marked reduction in the number of intercalated discs staining with the antibody. The granular brown material in the cytoplasm of the cells is lipofuscin; (*E*) Control immunostaining for plakoglobin.

Mutations in several other non-desmosomal genes have been linked with ARVC. These include: the ryanodine receptor 2 gene (RYR2);[38,39] mutations in the 5′ and 3′ untranslated regions of the transforming growth factor beta 3 gene;[40] the transmembrane protein 43 (TMEM43), a cytoplasmic membrane protein;[41] desmin, an intermediate filament protein[42,43] and, more recently, the titin gene.[44]

Lamin A and C are nuclear matrix proteins encoded by the same gene (*LMNA*) located on the nuclear surface of the inner nuclear membrane. *LMNA* mutations lead to a wide variety of clinical syndromes, but it is not clear how they result in such diverse phenotypes. Studies on transgenic *LMNA* mice have shown increased nuclear deformation, fragmentation of chromatin and abnormal mechanotransduction,[45,46] leading to impaired ability of the cell and nuclei to resist mechanical stress,[47,48] which has been proposed as a potential pathogenic mechanism in ARVC.[49]

As well as providing structural support to the cell, the nuclear envelope has an important role in the regulation of gene expression through the interaction of A-type lamins with signalling molecules, transcriptional regulators, and chromatin. While the relation between mutations in *LMNA* and the fibro-fatty change observed in this study requires further detailed examination, it is noteworthy that other laminopathies are characterized by alterations in adipose localization and replacement of skeletal myocytes by fibro-fatty tissue.[50,51] The observation of a reduction in plakoglobin staining in a single patient in this study is intriguing as the same

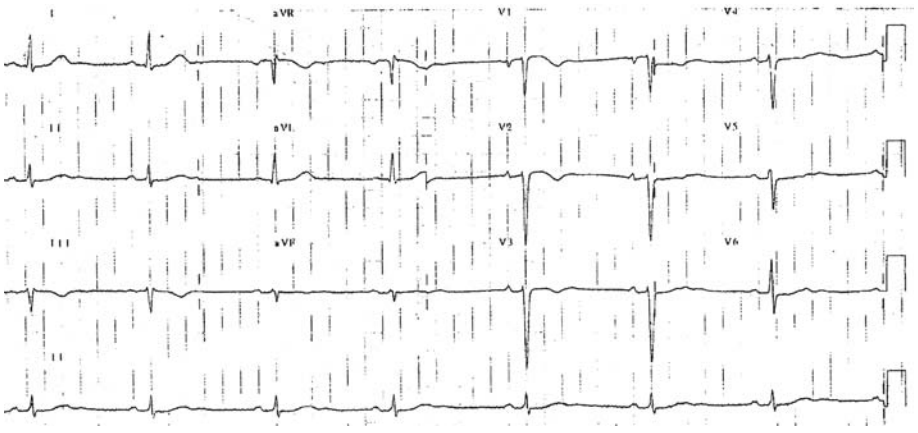

**Figure 5** Clinical characteristics of the nephew of patient D. Electrocardiogram shows sinus bradycardia, poor R-wave progression and T-wave inversion in V1–V3, and flat T-wave in V4–V5.

phenomenon is described in patients with ARVC caused by desmosomal protein gene mutations.[23] Studies using siRNA against desmoplakin[52] and an *in vitro* study of a human desmocollin mutation[53] suggest this is caused by abnormal localization of plakoglobin to the nucleus rather than reduced plakoglobin synthesis, but the reason for the dissociation of plakoglobin from the intercalated disc remains uncertain. The relation between abnormal plakoglobin localization and the pathophysiology of ARVC is similarly unclear. A number of studies have suggested that plakoglobin mislocalization is associated with a reduction in canonical Wnt/β-catenin signalling through Tcf/Lef1 transcription factors. Suppression of this pathway is known to cause adipogenesis, fibrogenesis, and apoptosis, the histological hallmark of the disease.

We speculate that abnormal interaction between lamin and emerin with Wnt/β-catenin,[54] and the adipogenic transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ) might explain the ARVC phenotype observed in this study. Adipogenesis is normally repressed via Wnt/β-catenin-dependent inhibition of PPAR-γ,[55] which promotes proteosomal degradation of β-catenin.[56] Interestingly, a similar interaction between plakoglobin and Wnt/β-catenin has been considered a possible pathogenic mechanism of ARVC.[52] As emerin and β-catenin reciprocally influence the other's expression and influence the onset of adipogenesis in cellular models of differentiating preadipocytes, it is possible that the ARVC phenotype in this study might arise because of the expansion and transdifferentiation of cardiac fibroblasts. Disturbance of Wnt signalling pathways could also be relevant to the reduced plakoglobin localization observed on immnunohistochemical staining, as plakoglobin competes with β-catenin in the nucleus causing a reduction in canonical Wnt/β-catenin signalling which normally inhibits adipogenesis.

More than one hundred *LMNA* mutations are described in patients with dilated cardiomyopathy.[19] The classical phenotype is that of early onset conduction system disease, atrial arrhythmias, and later progression to end-stage LV dysfunction.[10,57] Sudden cardiac death is common, even in those patients with pacemakers.[58] As the *LMNA* mutation carriers identified in this study had or went onto develop some of these features, it might be argued that these patients did not have classical ARVC but another overlapping phenotype. However, all the LMNA carriers fulfilled current task force criteria for ARVC at diagnosis and in the two patients examined, classical histological features. We therefore believe that LV involvement and conduction disease in this context should be considered as 'red flags' for suspecting a particular aetiological subtype of ARVC rather than a different diagnosis. The mechanisms responsible for this particular phenotype require further study, but the clinical implication is clear; namely that mutations in *LMNA* should be sought in patients with ARVC, particularly when they have conduction system disease or atrial arrhythmia. The small study cohort precludes definitive statements about management, but the poor outcome (including sudden cardiac death) in three of the four *LMNA* mutation carriers suggests that this form of ARVC should be managed in the same way as dilated cardiomyopathy caused by *LMNA* mutations with early consideration of ICDs and aggressive anti-failure medication.

Functional studies may have helped to clarify the pathogenic relevance of the LMNA variants found in our ARVC cohort, but this was beyond the scope of this study and further investigations to elucidate their pathogenic mechanism are needed.

## Conclusions

This study shows that *LMNA* mutations can be found in severe forms of ARVC and provides further evidence that it is not just a disease of desmosomal proteins. The major clinical implication is that LMNA should be added to desmosomal protein genes when performing genetic screening in patients with suspected ARVC, particularly when they also have ECG evidence for conduction disease.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

## Acknowledgements

This work is dedicated to Pietro Luigi Quarta and Teresa Carrozzo for their help and support.

## Funding

G.Q. is supported by a PhD Fellowship from the University 'La Sapienza' of Rome, by a European Society of Cardiology Research Fellowship and by InGenious Hypercare. W.J.M., P.S., and K.G. have been supported by EU 5th Framework Program Research & Technology Development—QLG1-CT-2000-01091, British Heart Foundation Program Grant RG/04/010. This work was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. Some of the work in this study was supported by a grant from the European Community Seventh Framework Programme (Theme Health-2009-2.4.2-3); Project title: INtegrated HEart Research In TrANslational genetics of dilated Cardiomyopathies in Europe (INHERITANCE).

**Conflict of interest**: none declared.

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