

Lamin A/C mutations associated with familial and sporadic cases of dilated cardiomyopathy in Koreans

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Abbreviations: ACE, angiotensin I-converting enzyme; DCM, dilated cardiomyopathy; FDC, familial dilated cardiomyopathy; LMNA, lamin A/C; LVEDD, left ventricular end-diastolic dimension

Abstract

Dilated cardiomyopathy (DCM) is characterized by cardiac dilation and systolic dysfunction. So far sixteen genes have been shown to cause autosomal dominant familial dilated cardiomyopathy (FDC). We identified a large Korean family from the Jeju island showing a clear Mendelian inheritance of FDC. A genomewide linkage scan at 9 cM marker density identified a peak multipoint LOD score of 2.82 at D1S195. Haplotyping of the region with 15 additional markers defined a candidate interval that included a known candidate gene encoding the lamin A/C (LMNA). Sequencing of the LMNA exons revealed one missense mutation at C568T (Arg190Trp) in the α -helical rod domain of the LMNA gene cosegregating with FDC with conduction-system disease. The same mutation was found in patients of another Korean family with FDC without conduction-system disease. Upon screening 14 sporadic DCM cases, we found three LMNA mutations including a case having a previously described (Glu161Lys) mutation and two having novel mutations (Glu53Val and

Glu186Lys). Our results suggest that variable genotypes of laminopathy are implicated in not only familial but also considerable proportion of sporadic DCM.

Keywords: Asian continental ancestry group; cardiomyopathy, dilated; genetics; heart conduction system; lamins; mutation

Introduction

Dilated cardiomyopathy (DCM) characterized by cardiac dilation and systolic dysfunction represents a heterogeneous group of inherited and acquired disorders (for review, see Ahmad *et al.*, 2005; Burkett and Hershberger, 2005). About 35% of individuals with DCM have a familial form of the disease (FDC) (Grunig *et al.*, 1998). FDC is highly heterogeneous disease genetically. Mutations in 25 chromosome loci have been described for DCM with or without additional clinical manifestations (for review, see Burkett and Hershberger, 2005). Among the 16 mutated genes associated with DCM, the human nucleoplasmic *lamin A/C* (*LMNA*) gene has been reported in forms associated with conduction-system disease with or without skeletal muscle myopathy.

In the present study we performed a linkage analysis followed by a candidate gene screening to localize the disease gene in a Korean family affected with an autosomal dominant severe DCM with conduction-system disease. By a mutation screen of the coding sequence of the *LMNA* gene on all family members, we identified a previously described missense mutation (Arg190Trp) in the rod domain of the *LMNA* gene that cosegregated with the disease in the family. We also found the same mutation in patients of another Korean family with FDC without conduction-system disease. Previously ten mutations (R60G, L85R, K97E, E111X, E161K, Arg190Trp, N195K, E203G, E317K, R377H) in the rod domain of the *LMNA* gene were reported to be associated with DCM through disorganization of the lamina (Fatkin *et al.*, 1999; Arbustini *et al.*, 2002; Sebillon *et al.*, 2003). Upon screening additional 14 patients, we found a previously described (Glu161Lys) mutation and two novel (Glu53Val and Glu186Lys) mutations. Our results show that *LMNA* mutations are associated with FDC and DCM in Koreans.

Materials and Methods

Clinical evaluations

We studied 35 subjects through 2 generations from one Korean family (family A), 2 subjects in another family (family B), and 14 sporadic cases without familial background. Six members of family A, the 2 tested patients in family B, and 14 sporadic cases showed phenotypically manifested DCM. The diagnosis of DCM was made based on previously accepted criteria (Mestroni *et al.*, 1999). Clinical evaluations included a detailed history, physical examination, 12-lead electrocardiogram, chest x-ray and echocardiogram.

Genotyping

Genomic DNA was isolated from peripheral blood samples following standard procedures (Lee *et al.*, 2001). Signed informed consent was obtained from all study subjects or their tutors according to the institutional review board policies. The genomewide scan was performed by using 392 highly polymorphic microsatellite markers spaced at an average distance of 9.0 cM at the McGill University and Genome Quebec Innovation Centre. Each marker was amplified individually followed by pooling of up to 8 markers together in panels. The genotyping results were obtained using an ABI-3700 or 3730

DNA Analyzer (Applied Biosystems). This study was approved by the Ethical Committee of the Asan Medical Center.

Linkage analysis

Two point linkage analyses were carried-out by using the MLINK routine of FASTLINK v4.1P. Multi-point linkage and haplotyping were done by using SIMWALK2 version 2.90 on Linux. For the genomewide scan, we used DNA samples from 25 pedigree members, of which six were from affected members. The pedigree provided sufficient power to detect linkage in a 10 cM scan with an estimated average linked LOD score of 1.12. The inheritance model was defined as dominant with 0.95 penetrance for pedigree members above age 45, 0.001 phenocopy rate, and a disease allele frequency of 0.001. All individuals younger than 45 years of age (except the spouses) were coded as phenotype unknown (Figure 1, family A) for the purpose of the linkage analyses. Marker allele frequencies were uniformly distributed to observed alleles counts due to the lack of reference samples for frequency estimation. Considering that the pedigree contains a high proportion of affected members, this should have a conservative effect on the LOD score statistics. Chromosome X was not considered for linkage due to the high number of male to male transmissions in the pedigree.

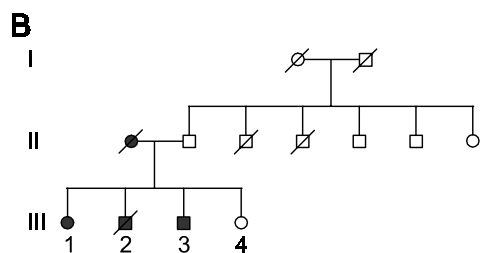
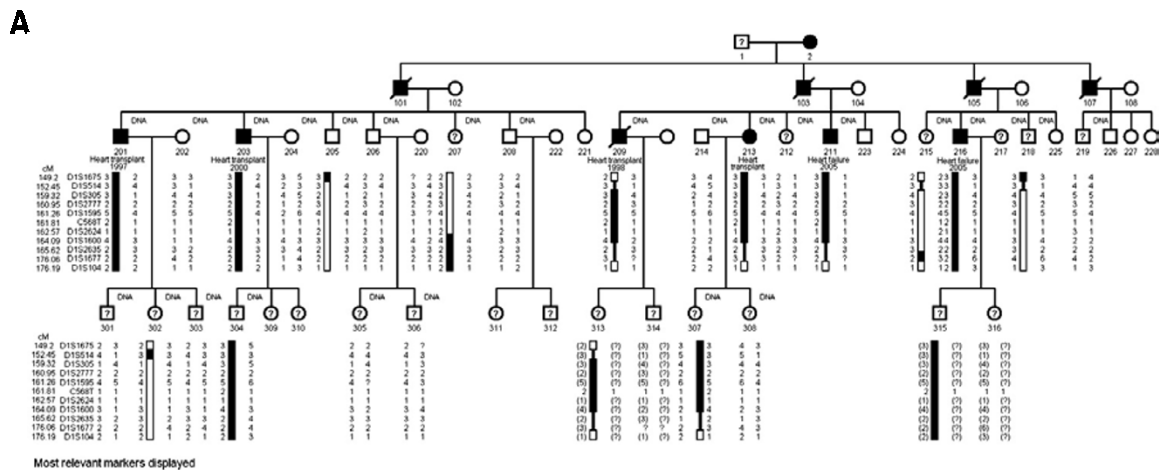


Figure 1. Pedigree of the families. Affected status is indicated by filled symbols, unaffected by clear symbols. Diagonal lines indicate deceased individuals. (A) Pedigree structure and haplotypes for family A. (B) Pedigree structure for family B.

Identification of mutations in the *LMNA* gene

All 12 exons and exon-intron junctions of the *LMNA* gene were amplified by PCR in the DNA sample from individual 201 of family A and were analyzed by direct sequencing using a MegaBACE 1000 sequencer (Amersham Biosciences, Buckinghamshire, England) following the manufacturer's instructions (primer sequences available upon request). One variant was identified in individual 201 from family A by sequence analysis of both strands. The presence

or absence of the sequence variant was ascertained in all 35 pedigree members from the family A, 2 siblings with FDC from family B, 14 additional sporadic cases, and 250 unrelated Korean controls by sequence analysis.

Results

Linkage analyses

The family with autosomal dominant inheritance of

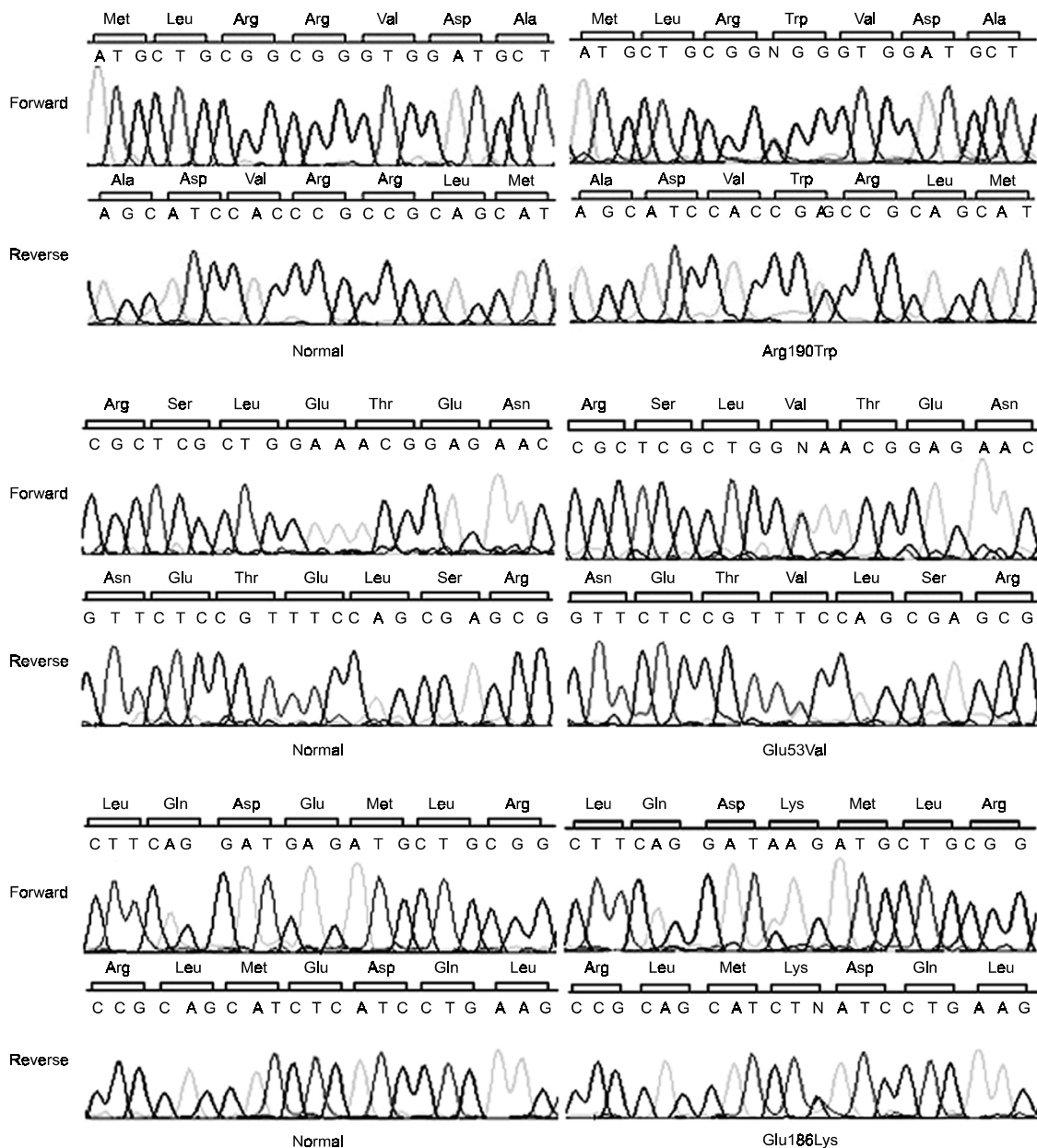


Figure 2. The direct sequencing results (both forward and reverse sequence) indicate the corresponding nucleotide substitutions.

DCM consisted of 4 generations including 2 living generations totaling 50 subjects and 29 subjects were fully genotyped (Figure 1A). There were no clinical data available from the deceased individuals of the 2nd generation of which all five males died in the fourth decades of life. Affected relatives were identified in 3 of 6 branches of the pedigree. Of 24 first-degree relatives, 5 males and one female were affected with idiopathic dilated cardiomyopathy. A genomewide screen was initiated, and a total of 392 microsatellite markers were genotyped. Five regions with LOD scores greater than 1 were found in two-point linkage on chromosomes 1, 2, 7, 13 and 21. Linkage to D1S1595 on chromosome 1 was strongly supported by the multipoint linkage with a LOD = 2.82, the highest finding in the study. Haplotyping over the chromosome 1 region revealed a candidate interval that included the known candidate gene, *LMNA*, encoding LMNA. To help resolve the difficulty in phase construction due to the unavailability of generations I and II and to define the recombinant boundaries for the candidate gene region, fifteen additional markers were added in the D1S1675 to D1S2107 interval. This allowed a 27 cM candidate interval (45.9 Mb) including the centromere and flanked by marker D1S1675 and D1S104.

Mutation analysis of a candidate gene

The *LMNA* gene was considered candidate for mutation in this family because the most frequent DCM-associated mutations have been reported in the *LMNA* gene located in chromosome 1q21.2. Twelve exon sequences of the *LMNA* gene were analyzed in proband, individual 201, of family A and a unique sequence variant which cause amino acid change at position 190 from Arg to Trp in exon 3 was identified (Figure 2). This mutation, Arg190Trp, was previously reported (Arbustini *et al.*, 2002; Anan *et al.*, 2002; Hermida-Prieto *et al.*, 2004) and predicted to alter residues in the α -helical rod domain of the peptide. Previously 10 missense mutations identified in the α -helical rod domain of the *LMNA* were reported to be disease-causing mutations in Caucasians with FDC and DCM. Then we also performed a mutation screen of the coding sequence of the *LMNA* gene on sibling samples with FDC from the other family and identified the same mutation, Arg190Trp (Figure 1B). By screening of additional 14 sporadic cases which showed nuclear detachment with evident cytoplasmic degeneration, we found three cases with *LMNA* mutations including a previously described (Glu161Lys) mutation and two novel mutations, Glu53Val and Glu186Lys (Figure 2). The fact that these variants were present in all the probands of the families and sporadic cases but

not in 504 chromosomes from normal individuals demonstrates that these variants are not a common polymorphism.

Clinical features of the *LMNA* mutation

There were 8 (6 from family A and 2 from family B) phenotypically manifested FDC patients from 2 families. The pedigrees are shown in Figure 1. The age at disease manifestation was early in middle age (mean 39 years; range 36-43) (Table 1). Five of 8 underwent heart transplantation (201, 203, 209, 213 of family A, III-3 of family B) because of advanced heart failure. They do well currently except individual 209 of family A who died. Mean time from the detection of heart failure to transplantation was 2.4 yrs (range 1.5-4 yrs). Three other patients (211, 216 of family A and III-1 of family B) are receiving ACE inhibitor and beta-blocker therapy for heart failure. Their initial ejection fraction and left ventricular end-diastolic dimension (LVEDD) were 24%, 6.1 cm, 34%, 6.6 cm, and 27%, 5.9 cm respectively.

In family A, 5 patients showed conduction-system disease as previously described (Fatkin *et al.*, 1999). They showed sinus-node dysfunction and/or disturbances in atrioventricular conduction (sinus bradycardia, bundle branch block and/or first-degree block). Atrial fibrillation/flutter which was paroxysmal atrial flutter was present in only one patient (213, family A). None of two patients in family B had any

Table 1. Clinical characteristics of 11 manifested dilated cardiomyopathy patients.

Patient ID	Sex/Age	Course	ECG	Af
Family A				
201	M/43*	Heart TPL	C-RBBB, 1° AVB	No
203	M/43*	Heart TPL	1° AVB, Sinus bradycardia	No
209	M/38*	Heart TPL	C-RBBB	No
213	F/40*	Heart TPL	C-RBBB, 1° AVB	Yes
211	M/40*	Medical Tx	Normal	No
216	M/37*	Medical Tx	Sinus bradycardia	No
Family B				
III-1	F/37*	Medical Tx	Normal	No
III-3	M/36*	Heart TPL	Normal	No
Sporadic cases				
Case 1	M/30	Heart TPL	C-LBBB	Yes
Case 2	M/42	Heart TPL	1° AVB, Sinus bradycardia	No
Case 3	M/42	Heart TPL	High grade AVB	Yes

Age*, age at clinical presentation (years old); TPL, transplantation; C-RBBB, complete right bundle branch block; C-LBBB, complete left bundle branch block; 1° AVB, the first degree Atrioventricular block; Af, atrial fibrillation; Medical Tx, medical treatment for heart failure

conduction-system disease.

There are 4 other family members in the 4th generation who have the same gene mutation but without phenotypically manifested familial dilated cardiomyopathy yet (304, 313, 307, 315 in Figure 1A). They do not show any conduction-system abnormalities. Their ages were 8, 16, 21 and 26 years.

None of family members including 8 phenotypically manifested DCM patients showed any evidence of skeletal muscle involvement such as skeletal muscle dysfunction, no weakness or wasting of skeletal muscle and no joint contracture. The serum creatine kinase level of all patients was in normal range.

All 3 patients of sporadic cases with LMNA mutation showed conduction-system disease (Table 1). In one patient with novel mutation (Glu53Val) was treated with permanent cardiac pacemaker due to high grade atrioventricular block at the age of 47 (case 3). The ages of onset were 30, 42 and 42 years. None showed skeletal muscle abnormalities. Two patients showed so rapid progression of heart failure that they underwent heart transplantation within 2 years. But in case 3, the progression was slow and he got heart transplantation after 8 years. In three families, no one else had heart failure.

Discussion

The pattern of inheritance of FDC includes autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance. Autosomal dominant form of FDC is the most frequent and can be grouped into either a pure DCM phenotype or DCM with conduction system disease. So far 25 genomic loci have been mapped in different DCM families, and 20 individual disease genes have been identified and screened for mutations in affected individuals (for review, see Burkett *et al.*, 2005). The genes involved in DCM encode a broad variety of proteins including sarcomeric, Z-disk-associated, sarcolemma cytoskeleton, intermediate filament, nuclear membrane, and calcium cycle proteins. Although numerous genes causing monogenic subtypes of DCM were identified, the extensive heterogeneity of disease phenotypes associated with mutations in a single gene or even in the same codon of that gene is not understood. It could be due to independent genetic factors or to environmental factors. Thus, we decided to carry out a genome-wide screen for an exclusion mapping.

The candidate region identified in genome-wide screen was flanked by markers D1S1675 and D1S104 in a 27 cM interval. From the region, Lamin A/C, a nuclear intermediate filament, is a best

candidate for FDC because it is well known that mutations in the *LMNA* gene cause a variety of human diseases including Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy and Hutchinson-Gilford progeria syndrome (Bonne *et al.*, 1999; 2003; Brodsky *et al.*, 2000; Raharjo *et al.*, 2001; Burke and Stewart, 2002). Furthermore, the most frequent DCM-associated mutations have been reported in the *LMNA* gene (Fatkin *et al.*, 1999; Brodsky *et al.*, 2000; Jakobs *et al.*, 2001; Arbustini *et al.*, 2002; Hershberger *et al.*, 2002; Sebillon *et al.*, 2003; Taylor *et al.*, 2003; Karkkainen *et al.*, 2004). DCM-associated mutations in the *LMNA* gene have been reported to cause very similar phenotypes characterized by atrial fibrillation, conduction system disease, ventricular dysrhythmia, need for pacemaker implantation, and high risk of sudden death. Recently, Taylor *et al.* (2003) showed that DCM patients with lamin A/C mutations had a poor prognosis compared with other DCM patients. They also found that lamin A/C mutations were associated with supraventricular arrhythmias, conduction-system disease, mild DCM (slightly dilated left ventricle), and the presence of skeletal muscle disease.

Eleven mutations of Lamin A/C associated with cardiac abnormalities are located in the central rod domain of the protein (Fatkin *et al.*, 1999; Arbustini *et al.*, 2002; Sebillon *et al.*, 2003). Of four mutations we found in the Korean families and sporadic cases, E53V and E186K were new missense mutations and the rest were not. The Arg190Trp mutation we found in the Korean families is previously described and reported to be located in the central rod domain (Anan *et al.*, 2002; Arbustini *et al.*, 2002; Hermida-Prieto *et al.*, 2004). This mutation has been associated with severe forms of familial DCM with conduction system disease but atrioventricular conduction defects were absent in the family with this mutation. Additional two mutations, E53V and E186K, we found in the two sporadic cases were also to be located in the central rod domain. Previously, it was shown that mutations of the *LMNA* gene frequently cause a particular phenotype DCM with conduction disease (Fatkin *et al.*, 1999; Brodsky *et al.*, 2000; Jakobs *et al.*, 2001; Taylor *et al.*, 2003; Karkkainen *et al.*, 2004). Subsequently a mouse line expressing *Lmna-N195K* variant of the A-type lamins with an asparagine-to-lysine substitution at amino acid 195 showed characteristics consistent with DCM with conduction system disease (Mounkes *et al.*, 2005).

In the Korean families we studied, the LMNA Arg190Trp mutation caused FDC in 2 families. This LMNA mutation in Korean families showed some different features. First, conduction-system disease was identified in 5 of 6 affected members in only one family. The same mutation did not cause conduction-

system disease in the other family. One member who had heart transplantation did not show any conduction-system disease even at the time of heart transplantation. The other one member detected heart failure 3 years ago but has not shown any conduction-system disease yet. Both families had DCM with rapid progression in young men, and the age of onset was also similar within each family. Second, our patients had the LMNA Arg190Trp mutation but atrioventricular conduction disturbance was present in 3 (38%) even though it was only first degree block and other conduction-system disease was present in the other 2 patients. Total 5 of 8 affected members (63%) had conduction-system disease. This figure is higher than previously reported (Anan *et al.*, 2002; Arbustini *et al.*, 2002). Third, the incidence of atrial fibrillation or flutter was low (12%, only 1 episode of paroxysmal atrial flutter in 1 patient) in our study patients. This figure is lower than that of previously described (59%) (Fatkin *et al.*, 1999).

All 3 sporadic cases had conduction-system disease and 2 showed atrial fibrillation. Based on our data, it is not clear whether mutations in LMNA are also responsible for conduction-system disease. However, our results support the convenience of using lamin A/C as a candidate gene in FDC with or without conduction abnormalities.

Since at least 30 to 40% of cases of DCM are inherited and FDC cannot be predicted on a clinical or morphological basis only, necessity of family screening is recognized by caregivers and scientists. However, it has also become clear that the disease is genetically and phenotypically highly heterogeneous. Multiple genes have been identified, most patients having private mutations, and the same mutation causing different phenotypes. The advances in the genetics of FDC will allow improved diagnosis, prevention and genetic counseling, and represent the basis for the development of new therapies.

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