

A novel *LMNA* indel mutation identified in a family with atrioventricular block and atrial fibrillation

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Abstract

It is well known that many genetic factors are involved in the occurrence and progression of atrioventricular block (AV block) and atrial fibrillation (AF). However, the genetic variants discovered so far have only explained parts of these processes. More genes and variants remain to be identified. In the present study, a three-generation family with an autosomal dominant form of AV block and AF was enrolled. Whole exome sequencing was conducted in three affected and one unaffected family member. A total of 64 nonsynonymous variants was shared by three affected individuals and not present in the unaffected individual. By selection of variants absent in the known databases and were predicted to be deleterious, 4 novel variants were identified. Only one novel frameshift insertion in the *LMNA* gene (c.825_826insCAGG) was identified in another affected family member and not detected in other non-affected family members and the 100 controls. Our finding expanded the spectrum of variants associated with AV block and AF, and was valuable in the genetic diagnosis of AV block and AF.

Abbreviations: AF = atrial fibrillation, AV block = atrioventricular block, CCD = cardiac conduction system disease, DCM = dilated cardiomyopathies, Indel = insertion/deletion, NMD = nonsense-mediated mRNA decay, PDGF = platelet-derived growth factor, PTC = premature termination codon, SNV = single-nucleotide variant, WES = Whole-exome sequencing.

Keywords: atrial fibrillation, atrioventricular block, mutation, pedigree

1. Introduction

Atrioventricular (AV) block, which represents a delay or disruption of the impulse transmission from the atrial to the ventricles, may result in syncope and sudden cardiac death. It is mainly due to an anatomical or functional impairment of the cardiac conduction system. About 40 percent of AV block cases are a result of ischemic heart disease. It is also associated with cardiomyopathies, cardiac surgery, medications, and inherited conditions.^[1]

Atrial fibrillation (AF) is the most common arrhythmia detected in clinical practice, and is associated with increased morbidity and mortality.^[2] Prior studies have raised the possibility that AV block may increase the risk of AF.^[3,4]

Although the pathophysiological processes underlying the occurrence and progression of AV block and AF are diverse, it is clear that many genetic factors are involved in the processes.^[5] To date, inherited arrhythmia has been found to be linked to genetic variants in genes coding for ion channels,^[6–9] cardiac transcription factors,^[10,11] or structural myocellular proteins.^[12] However, they only explain parts of these diseases and more genes and variants remain to be identified.

In the present study, we investigated a family containing four individuals with AV block, and three of them accompanied by atrial fibrillation. Whole-exome sequencing and Sanger sequencing were performed to explore the genetic background of this family.

2. Methods

2.1. Study subjects

A three-generation family from the North of China with an autosomal dominant form of AV block and AF was investigated. The pedigree of this family was shown in Figure 1. A completed questionnaire on health history, medical records, and blood samples were collected for each individual and available medical records were reviewed for deceased individuals. A control group of 100 inpatients aged ≥ 55 years old was recruited from Shanxi Cardiovascular Hospital in July 2018. They all had no records of cardiac conduction disease or atrial flutter or atrial fibrillation. This study was approved by the Ethics Committee of Shanxi Cardiovascular Hospital. Written informed consent was obtained from all subjects.

2.2. DNA extraction

Genomic DNA samples were isolated from peripheral EDTA-blood samples using the TIANamp Blood DNA Kit (TIANGEN,

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The datasets generated during and/or analyzed during the present study are not publicly available, but are available from the corresponding author on reasonable request.

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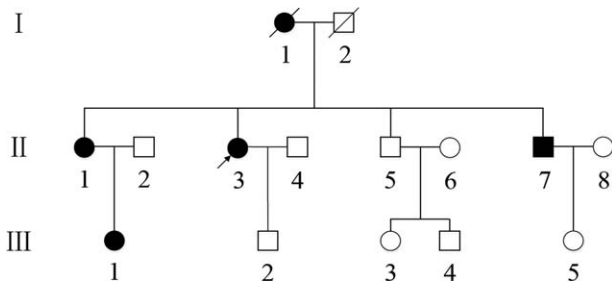


Figure 1. The pedigree of the family. Males and females are shown with squares and circles, respectively. Black circles/squares are affected, white is unaffected. Arrow indicates the proband.

Beijing, China) according to the manufacturer's standard protocol.

2.3. Whole exome sequencing

Whole-exome sequencing (WES) was performed for three affected individuals (II-1, II-3, and II-7 in Fig. 1) and one unaffected individual (II-5). The pre-capture library was constructed by the Agilent SureSelectXT Target Enrichment System for Illumina Paired-End Multiplexed Sequencing Library. Briefly, 200ng of genomic DNA was sheared into fragments of 150–200bp by the Covaris M220 System. Then the DNA fragments were subjected to the following steps: end-repair, adenylate 3' ends, and adapter ligation using the SureSelectXT Library Prep Kit. The adapter-ligated libraries were PCR amplified, followed by purification with AMPure XP beads. After the final purification, the quantity and size distribution of the PCR products was analyzed by Invitrogen Qubit 3.0 Fluorometer and Agilent 4200 TapeStation system with High Sensitivity D1000 Screen Tape respectively.

For exome capture, the prepared pre-capture libraries were individually hybridized to SureSelectXT Human All Exon Kit V6 using the SureSelectXT Reagent Kit. The hybridized DNA was captured using streptavidin-coated beads. Then the captured DNA libraries were subjected to the following steps: PCR amplification, purification, quantity and quality assessment.

After exome capture, the libraries were prepared for sequencing using Illumina's cBot cluster generation system. Then the flowcells were loaded to the Illumina HiSeq-X10 platform for paired-end sequencing.

2.4. Mapping, variant calling, and annotation

The data analysis was conducted using the GATK analysis pipeline:

1. the raw data in the format of fastq was mapped to the human reference genome hg19 with BWA v0.7.15 after the raw base quality assessment with FastQC.
2. Picard was used to marking the duplicates and calculate the base quality.
3. Local realignment and base quality recalibration were conducted with GATK v4.0.
4. single-nucleotide variant (SNV) and small insertion/deletion (indel) were detected with GATK Haplotype Caller.

5. SNV and indel annotation was performed with reference to dbSNP150, 1000Genomes, ESP6500, ExAC03, gnomAD, Hrcr1, and Kaviar_20150923.

2.5. Selection and exclusion

Variants detected in the GATK analysis were filtered according to the following criteria:

1. Variants within intronic, intergenic, UTR, upstream and downstream regions, as well as synonymous SNVs, were excluded;
2. Variants shared by three affected members and not present in the unaffected members were selected;
3. Variants present in the following databases: dbSNP150, 1000Genomes, ESP6500, ExAC03, gnomAD, Hrcr1, and Kaviar_20150923 were excluded;
4. SNVs which were not predicted to be deleterious by SIFT,^[13] PolyPhen2^[14] or Mutation taster^[15] were excluded;
5. SNVs with a Dann^[16] score <0.93 or a Cadd^[17] value <4 were further excluded.

2.6. Validation of candidate variants

To detect any possible false-positive errors and validate the candidate variants, Sanger sequencing was performed for the variants filtered from WES in the whole family members and controls.

3. Results

3.1. Clinical features

In this family, four members were affected with AV block (II-1, II-3, II-7, and III-1), and three of them were accompanied by AF (II-1, II-3, and II-7). The proband (II-3) is a 56 years old female who was healthy until age 39 when she had dizziness and palpitation, and the ECG showed second-degree type 2 AV block. Then she received a permanent cardiac pacing therapy. At age 51, she was diagnosed with AF, and a radiofrequency ablation therapy was received. The older sister and the younger brother of the proband (II-1 and II-7) were also diagnosed with AV block and AF. The family member III-1 was diagnosed with first-degree AV block by the routine physical examination at 28 years old. The clinical features of the affected family members are provided in Table 1 and Figure 2.

3.2. Whole exome sequencing outcomes

Whole-exome sequencing was conducted for three affected individuals (II-1, II-3, and II-7) and one unaffected individual (II-5), achieving a 99% coverage of the target region (Table 2). A total of 14,0745 variants were called by GATK, and 1082 of them which were nonsynonymous SNVs, splicing site mutations, and indels were selected for analysis (Fig. 3). Then the 64 variants shared by three affected individuals (II-1, II-3, and II-7) and not present in the unaffected individual (II-5) were selected because the disease is inherited in a dominant model in this family. After the exclusion of variants present in dbSNP150, 1000Genomes, ESP6500, ExAC03, gnomAD, Hrcr1 as well as Kaviar_20150923, 15 novel variants were identified. To predict the possible impact of the novel SNVs, SIFT, PolyPhen2,

Member	II-1	II-3(proband)	II-7	III-1
Sex	Female	Female	Male	Female
Current age(y)	58	56	53	34
Age at diagnosing(y)	46	39	41	28
Atrial-ventricular block	Third-degree AV block	Second-degree type 2 AV block	Third-degree AV block	First-degree AV block
Pacemaker	Yes	Yes	Yes	No
Atrial fibrillation	Yes	Yes	Yes	No
Radiofrequency catheter ablation	Yes	Yes	No	No

Mutation taster, Dann, and Cadd was used. SNVs which were not predicted to be deleterious by SIFT, PolyPhen2, or Mutation taster were excluded. SNVs with a Dann score <0.93 or a Cadd value <4 were also excluded. Then four candidate variants, including three nonsynonymous SNVs (*NUDT8*: exon2: c. 262G>C, p. E88Q, *TMC3*: exon22: c. 2896C>A, p. L966I, *KIF23*: exon9: c. 662G>A, p. S221N) and one frameshift insertion (*LMNA*: exon5: c. 825_826insCAGG, p. A274fs), were chosen for further co-segregation analysis.

3.3. Validation of candidate variants

Sanger sequencing was performed for the candidate variants in the whole family members and controls. The results of Sanger sequencing were consistent with WES in the four family members (II-1, II-3, II-5, and II-7). Among the four candidate variants, only one frameshift insertion in the *LMNA* gene (exon5: c.825_826insCAGG, p. A274fs) was identified in another

affected family member (III-1) and not detected in other non-affected family members (Fig. 4). Meanwhile, the insertion mutation was not found in the controls. Therefore, the frameshift insertion in the *LMNA* gene is the most likely candidate variant for this family.

4. Discussion

In the present study, we recruited a family with AV block accompanied by AF. By using WES and Sanger sequencing, we identified a frameshift insertion in the *LMNA* gene co-segregated with the phenotype in this family and didn't show in the controls. This indel mutation is a novel variant and has not yet been reported for any actual databases.

The lamin A/C (*LMNA*) gene is located on chromosome 1q22. It encodes two proteins, lamin A and lamin C, via alternative splicing of exon 10. Lamins are classified as intermediate filament proteins and are the major constituents of the nuclear lamina

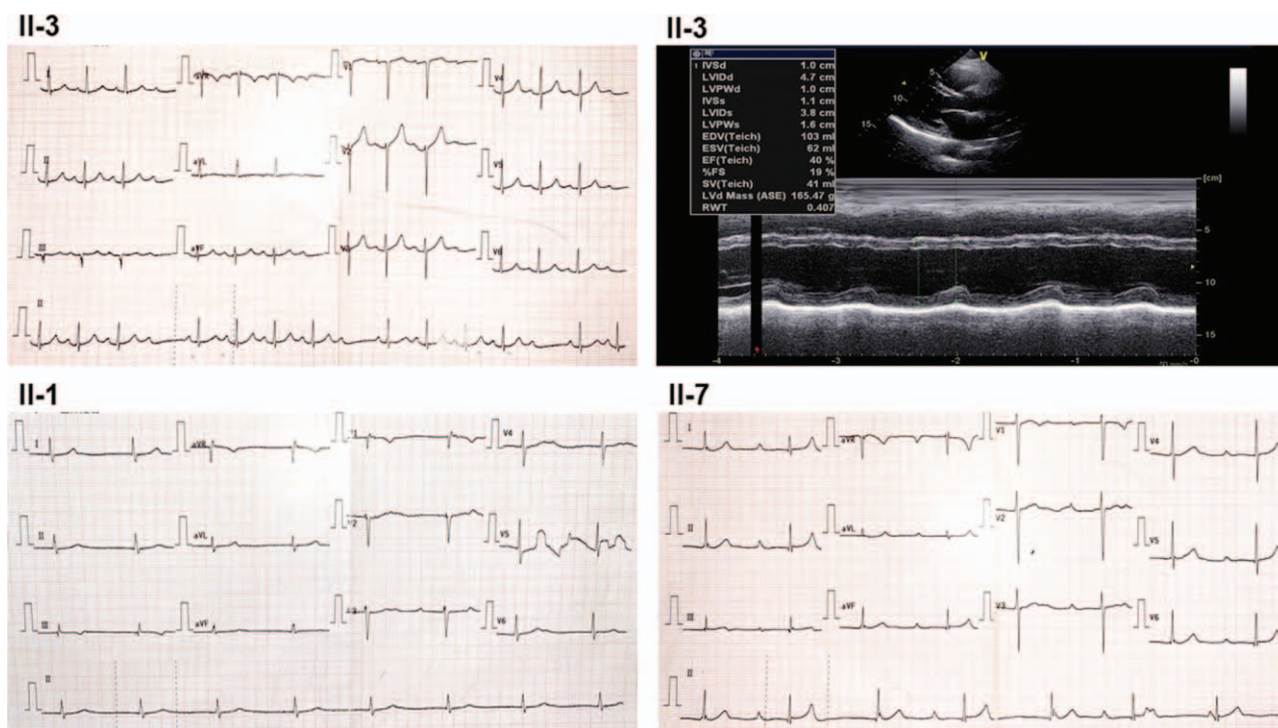


Figure 2. Clinical features of the affected family members. Electrocardiograms (ECGs) of the proband (II-3) showed second-degree type 2 AV block. ECGs of family member II-1 showed AF complicated with third-degree AV block. ECGs of II-7 showed third-degree AV block. Ultrasonic cardiogram (UCG) of the proband at 53 years showed decreased EF value.

Table 2
Summary of whole-exome sequencing statistics.

Member	II-1	II-3	II-5	II-7
Read length (bp)	150	150	150	150
Raw reads	289575166	308982474	327658264	280767344
Raw bases (bp)	43436274900	46347371100	49148739600	42115101600
Effective reads	289538046	308958051	327615978	280730934
Effective bases (bp)	43430706900	46343707650	49142396700	42109640100
Effective rate	0.9999	0.9999	0.9999	0.9999
Coverage of target region	0.9984	0.9979	0.9999	0.9998
GC%	0.5169	0.537	0.5171	0.5163
Q30	0.9213	0.9551	0.9175	0.9142
Q20	0.9688	0.9713	0.9668	0.9652
MEAN_TARGET_COVERAGE	211	224.97	239	204

MEAN_TARGET_COVERAGE: The mean read coverage of all target regions in an experiment.

which located next to the inner nuclear membrane.^[18] Lamins are considered to be involved in nuclear stability, chromatin organization, and gene expression.^[19]

More than 600 point-mutations in *LMNA* gene have been identified to be associated with a range of diseases, termed as laminopathies,^[19] including dilated cardiomyopathy with conduction disease, muscular dystrophies,^[20] lipodystrophies,^[21] neuropathies,^[22] and premature aging diseases.^[23] In the heart, *LMNA* mutations cause up to 10% of dilated cardiomyopathies (DCM),^[24] many coupled with progressive cardiac conduction system disease (CCD) or supraventricular arrhythmias. Prior

studies indicate that conduction system disease commonly precedes DCM development by a few years to a decade or more.^[25] In our study family, we found that the left and right atria of the proband were enlarged and the EF value was decreased slightly at age 53.

By inserting CAGG at position 825 of the cDNA sequence in *LMNA* gene, we found that the frameshift mutation creates a premature termination codon (PTC). Previous studies have shown that the mRNA that contains PTCs can be selectively degraded via nonsense-mediated mRNA decay (NMD).^[26,27] A study published on Nature in 2019^[28] had reported a

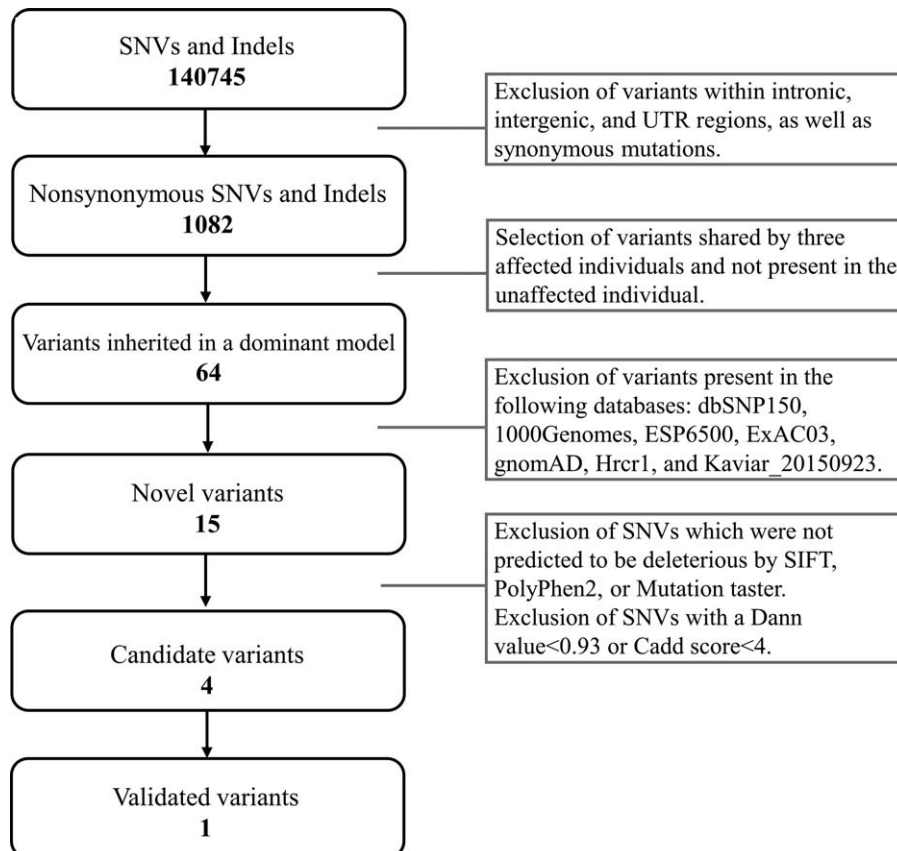


Figure 3. The flowchart of variants selection and exclusion.

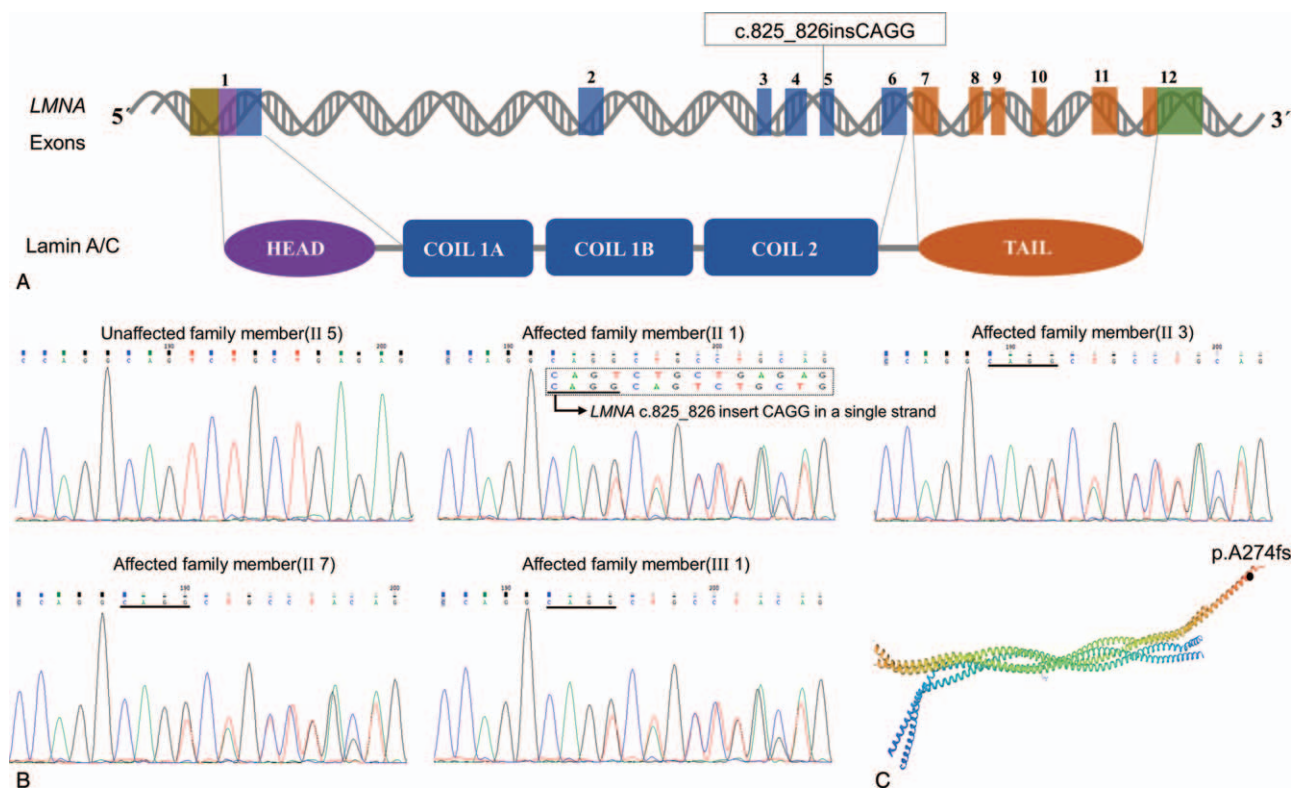


Figure 4. The 825_826insCAGG frameshift mutation in *LMNA* gene. (A) Schematic view of *LMNA* gene and the secondary structure of lamin A/C protein. (B) Sanger sequencing data for the 825_826insCAGG mutation in *LMNA* gene. (C) The crystal structure of the lamin A/C fragment (range: 27–282) predicted by Swiss-Model online.

348_349insG(K117fs) frameshift mutation in *LMNA* gene. It had found that the expression levels of *LMNA* mRNA and lamin A/C proteins were reduced in K117fs iPSC-CMs (induced pluripotent stem cell-derived cardiomyocytes). This study had explored the mechanisms that link *LMNA* mutations to arrhythmia, and found that aberrant calcium homeostasis is one of the links. It also found that the activation of the platelet-derived growth factor (PDGF) signaling pathway contributes to the pathogenesis of *LMNA* mutation induced arrhythmia.

There are two limitations, one is the present study lacks the genotype and phenotype information of the first generation family and the other is AV block patients with AF carrying the same indel would increase the credibility of the present study.

5. Conclusions

In conclusion, we found a novel frameshift insertion in the *LMNA* gene (c.825_826insCAGG) which is a pathogenic mutation leading to AV block and AF. Our finding expanded the spectrum of variants associated with AV block and AF, and would be helpful for the genetic diagnosis of AV block and AF.

Author contributions

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Supervision: Xiaoming Li.

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