



# Association of the *LMNA* gene single nucleotide polymorphism rs4641 with dilated cardiomyopathy

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**ABSTRACT.** Recently, studies on the pathogenesis of dilated cardiomyopathy (DCM) have focused on the underlying molecular biology and the association between single nucleotide polymorphisms (SNPs) and disease. This study was designed to explore the association between the rs4641 SNP of the *LMNA* gene and DCM in order to identify a new gene locus related to DCM. Polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing were employed to detect and genotype rs4641 in 198 patients with DCM and 160 healthy controls. Genotype and allele frequencies were compared to discover their relationship and logistic regression was used to assess the risk of DCM associated with the polymorphic variants. In the DCM group, the frequencies of the TC

and TT genotypes and the T allele of rs4641 were remarkably higher than those in the control group ( $P < 0.01$ ). According to risk analysis, taking the CC genotype as a reference, both the TC and TT genotypes increased the risk of DCM pathogenesis, with OR (95%CI) values of 5.957 (2.903-12.222) and 6.424 (2.156-19.141), respectively. Taking the C allele as the reference, presence of the T allele was found to increase DCM risk, with OR (95%CI) of 5.295 (3.121-8.983). These results suggested that the C to T mutation at the rs4641 locus of *LMNA* could enhance the risk of DCM, and that rs4641 represented a genetic susceptibility locus. Therefore, it was concluded that the *LMNA* rs4641 SNP was associated with DCM risk, which indicated that *LMNA* is a susceptibility gene for DCM.

**Key words:** Association analysis; *LMNA* gene; SNP; Dilated cardiomyopathy

## INTRODUCTION

Dilated cardiomyopathy (DCM) is one of the most common primary cardiac diseases, in which the left ventricular or, simultaneously, the left and right ventricular progressive expansion and contraction functions are reduced. DCM exhibits clinical phenotype diversity and genetic heterogeneity (Kim et al., 2014). The main clinical manifestations of DCM are heart failure, various arrhythmias, and cardiac embolism events, which might occur in the course of the disease at any time with a dismal prognosis (Yoshikawa et al., 2009). The onset of DCM is slow and this disease occurs mainly in men; it is rarely seen in women. The ages of onset in the population are between 20 and 50 years old, however, the age distribution becomes younger and the rate of this disease is increasing (Firuzi et al., 2013). At present, the pathogenesis of DCM remains unclear and the World Health Organization has defined DCM as a form of compound cardiomyopathy with involvement of both genetic and non-genetic factors (Maron et al., 2006). However, recent molecular genetic studies have confirmed the increasingly obvious primary role of genetic factors in the pathogenesis of DCM (Li et al., 2014; Matsa et al., 2014).

The *LMNA* gene is located on chromosome 1q21.2-q21.3 and is composed of 12 exons. *LMNA* produces the major lamin A and C proteins, and the minor A $\Delta$ 10 and C2 proteins by alternative splicing within exon 10, which are differentially expressed in a developmentally and tissue specific manner (Röber et al, 1989; Lin and Worman, 1993). At present, 458 different mutations from 2206 individuals have been identified in the *LMNA* gene ([www.umd.be/LMNA](http://www.umd.be/LMNA)). These mutations can be *de novo* or heritable, with a gain- or loss-of-function effect, and with severity ranging from minor arrhythmia arising in adolescence to a neonatal lethal tight skin condition (Zaremba-Czogalla et al., 2011). Lamin A/C is a structural protein of the nuclear envelope and cardiac involvement with Lamin A/C mutation was one of the first phenotypes to be reported in humans and suggested a crucial role of this protein in cardiomyocyte function (Carmosino et al., 2014). One study showed that approximately a third of DCM with atrioventricular block was caused by *LMNA* gene mutations, which indicated that the gene mutations could be considered as an important factor for DCM onset (Song et al., 2007). To date, numerous mutations within the *LMNA* gene locus have been suggested to lead to DCM (Taylor et al., 2003; Vytöpil et al., 2003; Kärkkäinen et al., 2004; Ben Yaou et al., 2005); however, the exact mechanism remains to be elucidated.

This study focused on the single nucleotide polymorphism (SNP) C1698T (rs4641) of the *LMNA* gene that represents a C-to-T nucleotide change located in the ninetieth base

of exon 10. Through discussing the correlation between SNP rs4641 and DCM, we aimed to identify the role played by SNPs of the *LMNA* gene in the process of DCM occurrence. We expected that this study would provide the relevant experimental basis and theoretical foundation for this determination.

## MATERIAL AND METHODS

### Study objects

#### *Patient group*

We selected 198 patients diagnosed with non-familial DCM in the Taishan Medical College Affiliated Hospital of Laiwu City People's Hospital from June 2011 to March 2014 for this study. These consisted of 152 men and 46 women, from 19 to 85 years of age with a mean age of  $54 \pm 11.5$  years. According to the DCM diagnostic criteria established by the World Health Organization and the International Association of Cardiology in 2006, diagnosis exclusion criteria included any of the following items: hypertension, coronary heart disease, valvular heart disease, cardiac disease, perinatal cardiomyopathy, clear history of viral myocarditis, endocrine and metabolic disorders (such as diabetes, pheochromocytoma, thyroid disease, Cushing's syndrome), systemic disease (sarcoidosis, autoimmune disease), tumor, skeletal muscle disease, or a long history of heavy drinking.

#### *Control group*

A total of 160 healthy controls randomly selected from hospital outpatient clinics included 118 men and 42 women, from 15 to 84 years with a mean age of  $52 \pm 15.1$  years. No statistical differences existed in age or gender between the patient and control groups ( $P < 0.05$ ).

All participants in both groups voluntarily joined this study and signed informed consents. This research was approved by the Clinical Trials and Biomedical Ethics Committee of the Taishan Medical College Affiliated Hospital of Laiwu City People's Hospital.

### Experimental methods

#### *Genomic DNA extraction*

We extracted 3mL fasting venous blood from all patients and healthy controls. We used 0.5 mM ethylenediaminetetraacetic acid to keep the blood from clotting. Genomic DNA was extracted using standard phenol-chloroform DNA extraction methods.

#### *Primer design*

The nucleotide sequence of exon 10 of the *LMNA* gene was obtained from GenBank (Gene ID: 4000, accession No. NC\_000001.11). Polymerase chain reaction (PCR) primers were designed by the Primer Premier 5.0 software (<http://www.premierbiosoft.com/primerdesign/>) and synthesized by Shanghai Sangon Biological Co., Ltd. (Shanghai, China). Primer sequences were as follows: forward 5'-TGCTGTACAACCCTCCCTGG-3', reverse: 5'-GGGTTCCCTGTTCAA GGTATA-3'.

### **PCR-restriction fragment length polymorphism (RFLP)**

The PCR system totaled 25  $\mu$ L and contained 20 ng DNA template, 1  $\mu$ L 10 pM forward and reverse primers respectively, 2.0  $\mu$ L 2.5 mM dNTP mixture, 12.5  $\mu$ L 2X GC buffer, 0.2  $\mu$ L 5 U/ $\mu$ L LA-Taq DNA polymerase (BioTeke Corporation, Beijing, China), and 7.3  $\mu$ L ddH<sub>2</sub>O. PCR was carried out as follows: denaturation at 94°C for 5min, followed by 32 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR product was digested with *Pm1*I endonuclease at 37°C in a water bath for 4 h. Then, the digested samples were detected by 1.8% agarose gel electrophoresis. According to the observed cleavage pattern of the DNA fragments, the *LMNA* genotype was defined as CC, TT, or CT.

### **Sequence identification**

A fraction of the PCR product was sent to Invitrogen Corporation (Carlsbad, CA, USA) for sequencing. The DNAMAN software (<http://www.lynnon.com/>) was adopted to produce alignments of the sequencing results and the templates.

### **Statistical analysis**

The proportions of genotype and allele frequencies among the patients and controls were calculated by a direct counting method. Data analysis was performed by adopting the SPSS17.0 software (SPSS, Chicago, IL, USA). The  $\chi^2$  test was employed to compare genotype and allele frequencies among groups. Logistic regression was adopted to perform risk analysis. Statistical significance was defined as  $P < 0.05$ .

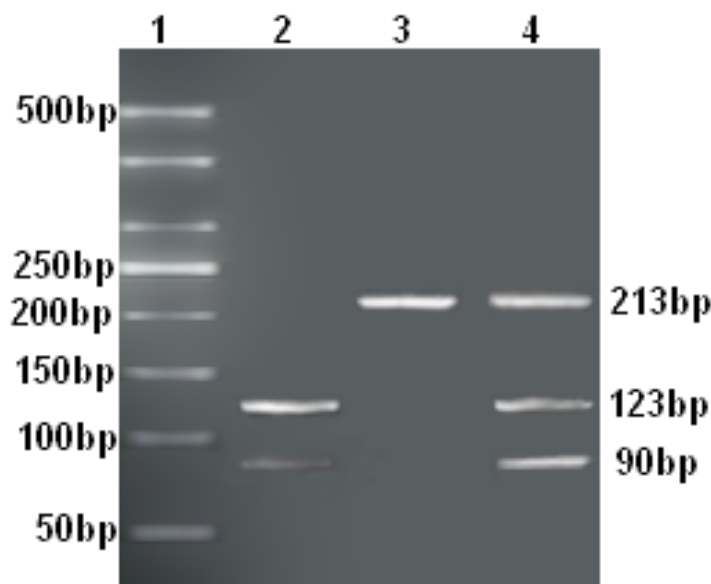
## **RESULTS**

### **Genotyping**

From the results of PCR-RFLP (Figure 1) and sequencing, it could be seen that three genotypes could be identified for the *LMNA* rs4641 loci: CC, CT, and TT. CC homozygotes carried the *Pm1*I restriction site (CAC|GTG) and could produce DNA fragments of 123 and 90 bp after enzyme digestion. CT heterozygotes also had this site at one locus and produced three DNA fragments: 213, 123, and 90 bp. TT homozygotes had no such site and only produced 213 bp DNA fragments.

### **Test for goodness of fit**

In order to confirm whether the rs4641 genotype frequencies of the samples conformed to Hardy-Weinberg equilibrium, a test for goodness of fit was performed, respectively, in the DCM and control groups and in a combination of the two groups (combination group). The results showed that the expected values of genotype coincided best with the observed values in these three groups, indicating that the genotype distribution in both the DCM and control groups accorded with Hardy-Weinberg equilibrium and samples were representative of the population (Table 1).



**Figure 1.** Detection of *LMNA* gene polymorphism by PCR-RFLP. *Lane 1:* 50-bp DL 2000 Marker; *Lane 2:* CC genotype; *Lane 3:* TT genotype; *Lane 4:* CT genotype. PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

**Table 1.** Hardy-Weinberg equilibrium test of rs4641.

Genotype	DCM group (N = 198)				Control group (N = 160)				Combination group (N = 358)			
	Observed value	Expected value	$\Sigma\chi^2$	P	Observed value	Expected value	$\Sigma\chi^2$	P	Observed value	Expected value	$\Sigma\chi^2$	P
CC	125	118			146	148			271	266		
TC	51	48			10	8			61	56		
TT	22	32	0.108	0.948	4	4	1.546	0.989	26	36	0.023	0.462

### Association analysis between SNP rs4641 and DCM

The genotype frequencies of CC, TC, and TT in the DCM group were 63.13, 25.76, and 11.11%, respectively, and in the control group were 91.25, 6.25, and 2.5%. The difference of the genotype distributions between the two groups was of statistical significance ( $\chi^2 = 63.533$ ,  $P = 0.000$ ). Taking the CC genotype as the reference, the risk of DCM in patients with the TC and TT genotypes was increased ( $P < 0.01$ ), and the relative risk ratios were 5.957 (2.903-12.222) and 6.424 (2.156-19.141) (Table 2).

**Table 2.** Distribution of genotypes in rs4641 locus.

Genotype	DCM group(%)	Control group(%)	$\chi^2$ value	P	OR (95%CI)
CC	125 (63.13)	146 (91.25)	-	-	1
TC	51 (25.76)	10 (6.25)	28.082	0.000	5.957 (2.903-12.222)
TT	22 (11.11)	4 (2.5)	14.060	0.000	6.424 (2.156-19.141)
TC + TT	73 (36.87)	14 (8.75)	38.034	0.000	6.090 (3.277-11.318)

$\chi^2 = 63.533$ ,  $P = 0.000$

Distributions of the rs4641C and T alleles between the DCM and control groups were remarkably different and the difference was significant ( $\chi^2 = 44.910$ ,  $P = 0.000$ ). The T allele frequency (23.99%) in the DCM group was apparently higher than that in the control group (5.63%). The T allele significantly increased the risk of DCM with OR (95%CI) of 5.295 (3.121-8.983) (Table 3).

**Table 3.** Distribution of alleles in rs4641 locus.

Allele	DCM group (%)	Control group (%)	$\chi^2$	P	OR (95%CI)
C	301 (76.01)	302 (94.38)			
T	95 (23.99)	18 (5.63)	44.910	0.000	5.295 (3.121-8.983)

## DISCUSSION

Fatkin et al. (1999) first reported the connection between *LMNA* gene mutation and DCM combined with disorders of the cardiac conduction system. According to some statistics, the pathogenic gene underlying approximately 5% of familial DCM is *LMNA*, which is identified as DCMIA and exhibits autosomal dominant inheritance. The main presentation of familial DCM is DCM with abnormalities of cardiac conduction system that is related to age. In addition, DCM is associated with abnormal electrophysiology caused by conduction disturbances of the sinoatrial and atrioventricular nodes, among others (Perrot et al., 2009). DCM caused by *LMNA* gene mutation is characterized by early onset of atrioventricular block prior to the manifestation of DCM; accordingly, high sudden death rate and malignant arrhythmia are common causes of death (Taylor et al., 2003; Kärkkäinen et al., 2004). Many scholars believe that DCM caused by *LMNA* gene mutation has a worse prognosis than do other etiologies (Mercuri et al., 2005; Sylvius et al., 2005). However, the pathogenesis of DCM remains unknown.

In recent years, as increasing amounts of research on DCM is performed, approximately 40 *LMNA* gene mutation loci have been found to cause DCM associated with abnormal conduction system; these are distributed over the 12 exons of the gene (Mercuri et al., 2005). Mutations E161K and R377H could result in the inability of the lamin A protein monomer to polymerize into nuclear lamina protein (Sébillon et al., 2003). Arbustini et al. (2002) found that K97E, E111X, R190W, and E317K were related to DCM, and their results suggested that approximately 33% of patients with DCM and atrioventricular block carried an *LMNA* gene mutation. Results of the research of Kärkkäinen et al. (2004) showed that the S143P mutation played a marked role in the occurrence of DCM. Hershberger et al. (2002) investigated a genealogy with DCM and found that approximately 40% of the members carried the L215P mutation on exon 4, although analysis of *LMNA* gene mutation function could not completely clarify the mechanism by which this variant led to DCM. Overall, the presence of SNPs in *LMNA* is expected to become a clinical predictor of DCM.

Correlation between the C1698T polymorphism of the *LMNA* gene and DCM has not previously been reported. Here, this study detected the genotypes of C1698T (rs4641) in exon10 of *LMNA* in 358 participants using PCR-RFLP and gene sequencing. Results showed that although C1698T is a silent mutation and does not cause an amino acid change, the TC and TT genotype and the T allele frequencies at the C1698T loci of patients with DCM were obviously higher than those of healthy controls. Using the CC genotype as the reference, the CT and TT genotypes could significantly increase the risk of DCM onset and the T allele was determined to convey disease risk. Together, these findings indicated that this locus polymorphism might have a certain correlation with the risk of DCM. A potential mechanism for this effect might be that the C1698T variant, located on

exon 10, could produce both lamin A and lamin C by alternative splicing. Therefore, C1698T might affect selective alternative splicing and lead to changes of mRNA content and protein levels.

Genetic factors play an important role in the occurrence and development of DCM and it is of importance to identify the etiology and pathogenesis of DCM at the gene level. Detection of SNPs represents a new strategy for the diagnosis and therapy of DCM that will facilitate early diagnosis, early prevention, and individualized treatment of this disorder.

## Conflicts of interest

The authors declare no conflict of interest.

## REFERENCES

- Arbustini E, Pilotto A, Repetto A, Grasso M, et al. (2002). Autosomal dominant dilated cardiomyopathy with atrioventricular block: a lamin A/C defect-related disease. *J. Am. Coll. Cardiol.* 39: 981-990.
- Ben Yaou R, Muchir A, Arimura T, Massart C, et al. (2005). Genetics of laminopathies. *Novartis Found. Symp.* 264: 81-90.
- Carmosino M, Torretta S, Procino G, Gerbino A, et al. (2014). Role of nuclear lamin A/C in cardiomyocyte functions. *Biol. Cell.* 106: 346-358.
- Fatkin D, MacRae C, Sasaki T, Wolff MR, et al. (1999). Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease. *N. Engl. J. Med.* 341: 1715-1724.
- Firuzi O, Shakibzad N, Amoozgar H, Borzooee M, et al. (2013). Effects of omega-3 polyunsaturated fatty acids on heart function and oxidative stress biomarkers in pediatric patients with dilated cardiomyopathy. *Int. Cardiovasc. Res. J.* 7: 8-14.
- Hershberger RE, Hanson EL, Jakobs PM, Keegan H, et al. (2002). A novel lamin A/C mutation in a family with dilated cardiomyopathy, prominent conduction system disease, and need for permanent pacemaker implantation. *Am. Heart J.* 144: 1081-1086.
- Kärkkäinen S, Heliö T, Miettinen R, Tuomainen P, et al. (2004). A novel mutation, Ser143Pro, in the lamin A/C gene is common in Finnish patients with familial dilated cardiomyopathy. *Eur. Heart J.* 25: 885-893.
- Kim JH, Kim SY and Park JH (2014). Dilated cardiomyopathy with left ventricular thrombi as a presenting feature of Cushing disease. *Can. J. Cardiol.* 30: 1462.e11-3.
- Li J, Liu WD, Yang ZL, Yuan F, et al. (2014). Prevalence and spectrum of *GATA4* mutations associated with sporadic dilated cardiomyopathy. *Gene* 548: 174-181.
- Lin F and Worman HJ (1993). Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. *J. Biol. Chem.* 268: 16321-16326.
- Maron BJ, Towbin JA, Thiene G, Antzelevitch C, et al. (2006). Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 113: 1807-1816.
- Matsa LS, Sagurthi SR, Ananthapur V, Nalla S, et al. (2014). Endothelin 1 gene as a modifier in dilated cardiomyopathy. *Gene* 548: 256-262.
- Mercuri E, Brown SC, Nihoyannopoulos P, Poulton J, et al. (2005). Extreme variability of skeletal and cardiac muscle involvement in patients with mutations in exon 11 of the lamin A/C gene. *Muscle Nerve* 31: 602-609.
- Perrot A, Hussein S, Ruppert V, Schmidt HH, et al. (2009). Identification of mutational hot spots in *LMNA* encoding lamin A/C in patients with familial dilated cardiomyopathy. *Basic Res. Cardiol.* 104: 90-99.
- Röber RA, Weber K and Osborn M (1989). Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. *Development* 105: 365-378.
- Sébillon P, Bouchier C, Bidot LD, Bonne G, et al. (2003). Expanding the phenotype of *LMNA* mutations in dilated cardiomyopathy and functional consequences of these mutations. *J. Med. Genet.* 40: 560-567.
- Song K, Dubé MP, Lim J, Hwang I, et al. (2007). Lamin A/C mutations associated with familial and sporadic cases of dilated cardiomyopathy in Koreans. *Exp. Mol. Med.* 39: 114-120.
- Sylvius N, Bilinska ZT, Veinot JP, Fidzińska A, et al. (2005). *In vivo* and *in vitro* examination of the functional significances of novel lamin gene mutations in heart failure patients. *J. Med. Genet.* 42: 639-647.
- Taylor MR, Fain PR, Sinagra G, Robinson ML, et al. (2003). Natural history of dilated cardiomyopathy due to lamin A/C gene mutations. *J. Am. Coll. Cardiol.* 41: 771-780.

- Vytopil M, Benedetti S, Ricci E, Galluzzi G, et al. (2003). Mutation analysis of the lamin A/C gene (*LMNA*) among patients with different cardiomyopathic phenotypes. *J. Med. Genet.* 40: e132.
- Yoshikawa T, Baba A and Nagatomo Y (2009). Autoimmune mechanisms underlying dilated cardiomyopathy. *Circ. J.* 73: 602-607.
- Zaremba-Czogalla M, Dubińska-Magiera M and Rzepecki R (2011). Laminopathies: the molecular background of the disease and the prospects for its treatment. *Cell. Mol. Biol. Lett.* 16: 114-148.