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Original Article

LMNA gene single nucleotide polymorphisms in dilated cardiomyopathy of Han children

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Abstract: Objective: To investigate whether LMNA gene mutation is associated with dilated cardiomyopathy (DCM) in Chinese Han Race children. Methods: DNA was isolated from 78 patients with DCM and 100 healthy Chinese children who served as controls. 12 exons in the functional regions and the adjacent part of introns of the LMNA gene were amplified with polymerase chain reactions (PCR) and the PCR products were sequenced with DNA sequencer. We compared the DNA sequence with Blast software online PubMed website. The differences of allele and genotype between the groups were detected by χ^2 test. Results: No disease-causing mutation in LMNA gene was found in all DCM patients. Three nonsense single nucleotide polymorphisms (SNPs) were identified. ① The first is c.1908C>T (H566H, rs4641) which was located at exon 10 of LMNA gene. It was found in 29 DCM cases and 15 control subjects. Compared to healthy controls, the frequency of TT and TC genotypes, and the C allele were significantly increased in DCM patients ($P<0.05$). ② The second was c.861C>T (A287A, rs5380) which was located at exon 5 of LMNA gene. It was found in 9 DCM cases and 2 control subjects. The frequency of TC genotype was significantly increased in DCM patients ($P<0.05$). ③ The third was c.1338C>T (D446D, rs5058) which located at exon 7 of LMNA gene. It was found in 8 DCM cases and 3 control subjects. The frequency of TC genotype was significantly increased in DCM patients ($P<0.05$). Conclusion: The SNP of LMNA gene may be associated with the susceptivity of DCM in Chinese Han children.

Keywords: Dilated cardiomypathy, LMNA gene, single nucleotide polymorphisms

Introduction

Dilated cardiomyopathy (DCM) is a disease of the heart muscle characterized by ventricular dilatation and impaired systolic function [1]. Morbidity of DCM is appoximately 5 to 8/100 thousand, prevalence rate of DCM is about 36.5/100 thousand [2]. The average age of children DCM is 1.5 years old, mortality rate for the onset age before 5 years old is at 15 to 50%. So DCM is a leading cause of heart failure and arrhythmia [3]. DCM patients accounted for 3.79% in pediatric cardiology ward and 55.2% in cardiomyopathy patients in a retrospective study in Shanghai Children's Medical Center Affiliated to Shanghai Jiaotong University School of Medicine in 2009 [4]. And the DCM patients 5 years mortality was 45.5% [4]. Due to its significant prevalence, high mortality and morbidity, including frequent hospitalizations, DCM is a major health concern both in the pediatric and adult population.

It is reported that 20 to 50% of DCM patients have a family history [1]. Now it has been found more than 20 chromosomal loci associated with its mode of inheritance is autosomal dominant majority. LMNA gene which is located on chromosome 1q21.2-1q21.3 encodes lamin protein (lamin A/C). LMNA gene has 12 exons and the adoption of exon 10 alternative splicing produces both lamin A and lamin C mRNA. LMNA gene mutations can cause many diseases, including DCM. Now LMNA mutation has been thought it is one of the important factors which cause DCM [5]. So we study LMNA gene mutation in Chinese Han children with DCM to explore the role of LMNA gene mutation in the pathogenesis of DCM.

Materials and methods

Subjects and grouping

We collected 78 children with DCM in Shanghai Children's Hospital and Shanghai Children's

Table 1. DCM patients' clinical phenotype

Phenotype	Number	Phenotype	Percent (%)
Primary clinical exhibition	78	Chest X-ray	
Face edema	17	pulmonary congestion	65.7
Tired after exercise	19	cardiothoracic ratio	62
Palpation	22		
Cardiac shock	4		
Others	14		
Electrocardiogram	78	echocardiography	
Atrial extrasystole	13	LVEF (%)	40
Atrial tachycardia	5	LVFS (%)	23
Atrial flutter	3		
Atrial fibrillation	5		
Ventricular extrasystole	4		
AV block	15		
Others	33		

Table 2. Primers of LMNA gene 1-12 exons and PCR products

Exon	Primer (5'→3')	Primer (3'→5')	PCR product (bp)
1	tctctgtccttcgacccgag	cctctccactccccgcca	490
2	ctggtaattgcaggcatagc	ttacaggcgtgaaccaccat	550
3	acctctcagcttccttccagtt	ctagcccagcccaagtctgt	250
4	gcctcccaggaactaattctg	cgtgggtaagggtagggctg	280
5	atgcccaactcaggcctgtg	gcttccagcctgcatccgg	250
6, 7	tccctccttccccatacttag	ccctgatgcagctgtatcccc	640
8, 9	caagatacacccaagagcctg	ctcgtccagcaagcagccag	420
10	tgctgtacaacccttccctgg	gggttccctgttcaaggtata	320
11	gttgggcctgagtggtcag	cacctcgtcctacccctcg	400
12	ggctggagtgtgagggatg	cctcccatgacgtgcaggg	220

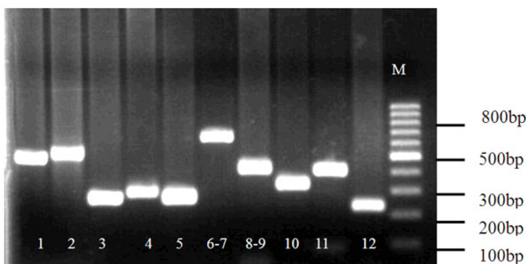

Figure 1. PCR product of 12 extrons of LAMN. M means DNA marker; 1~12 means different LMNA extron.

Medical Center Affiliated to Shanghai Jiaotong University School of Medicine from January 2009 to October 2010. The DCM patients were 41 males and 37 females and the mean age was 7.7±5.4 years old (3 months to 16 years old). All patients were diagnosed with the criteria of 1995 WHO/International Society and Federation of Cardiology (ISFC) Task Force on the Definition and Classification of the Cardiomyopathies [6]. All the objects were confirmed by medical examination, medical history, 12-lead electrocardiogram, transthoracic echocardiography, chest X ray and other tests. The patients' detail data were described in **Table 1**. 100 healthy children were collected as control group, which including 54 males and 46 females, aged 1 year to 18 years old, mean age was 6.5±3.2 years old. The study was approved by the hospital ethics committee and all subjects were informed consent to participate in this study voluntarily.

DNA Extraction and polymerase chain reaction (PCR)

2 ml peripheral blood was obtained from all subjects and high molecular weight genomic DNA was isolated by established methods [7]. Protein-encoding sequences from exons 1 through 12 were amplified from genomic DNA with the use of primers derived from intron sequences (**Table 2**). The sequences are available on the Internet (at http://genetics.med.harvard.edu/~seidman/lamin.html). *PCR conditions were denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 sec, annealing at 55-58°C for 45 sec and extension at 72°C for 30 sec, with final extension at 72°C for 10 min.

DNA sequencing

Genomic DNA fragments amplified with the PCR were purified with a PCR purification kit to remove the residual primers and sequenced with a dye-terminator cycle-sequencing system (ABI PRISM 3730) in Shanghai Huada Gene Corporation. We compared the DNA sequence with the normal LAMN gene with Blast software in PubMed.

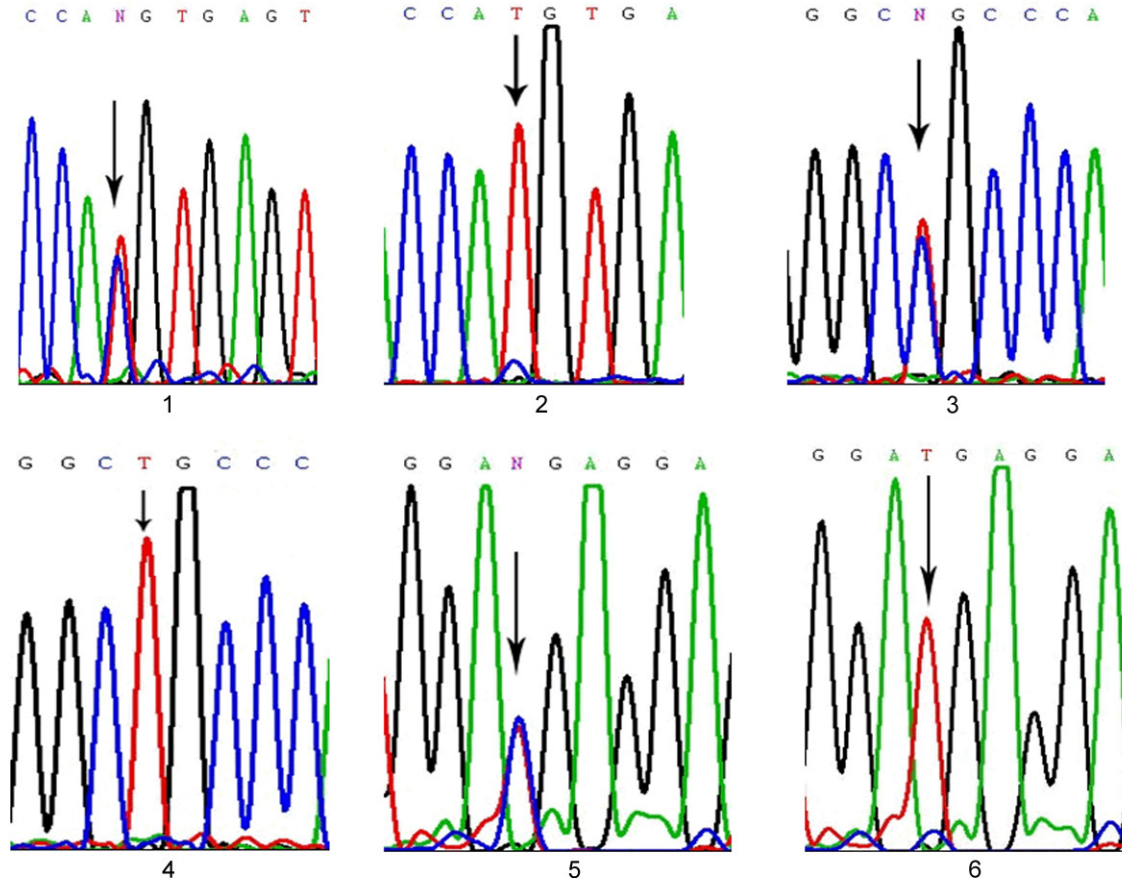

Figure 2. SNPs of T1908C, T861C and T1338C in LMNA gene. 1, 3, 5 mean T1908C, T861Cand T1338C in DCM and 2, 4, 6 mean T1908T, T861T and T1338T in control. All SNPs were confirmed by reverse sequencing.

Statistical methods

The test group and control group genotype frequency distribution were used Hardy-Weinberg balance test samples to identify group representative. The measurement data were given as mean value ± SD. Direct count method to calculate gene frequency and genetic type such as frequency, and genotype frequencies; group allele and gene difference were used the chi-square test ($P<0.05$ means difference has statistical significance). The SPSS16.0 statistics analysis software was used to process data.

Results

In this study, all 12 exons of the LMNA gene were screened for variants in a total of 78 DCM patients and 100 control samples by the PCR method (**Figure 1**). The LMNA gene mutation was not found in all DCM patients and control samples. Three nonsense single nucleotide polymorphisms (SNPs) were identified. One was T1908C (rs4641) which was located at exon 10 of LMNA gene. It was found in 29 DCM cases and 15 control subjects. Compared to healthy controls, the frequency of TT and TC genotypes and the C allele were significantly increased in DCM patients ($P<0.05$) (**Figure 2**; **Table 3**). The second SNP was T861C (rs5380) which was located at exon 5 of LMNA gene. It was found in 9 DCM cases and 2 control subjects. The frequency of TC genotype was significantly increased in DCM patients ($P<0.05$) (**Figure 2**; **Table 3**). The third SNP was T1338C (rs5058) which located at exon 7 of LMNA gene. It was found in 8 DCM cases and 3 control subjects. The frequency of TC genotype was significantly increased in DCM patients ($P<0.05$) (**Figure 2**; **Table 3**).

Discussion

With the progress of diagnostic methods include ultrasound, CT and MRA in recent years, the morbidity and mortality of DCM are

Table 3. Genotype of T1908C, T861C and T1338C in DCM and control

SNPs	group	case number	genotype number		χ^2	*P*
			C allele	T allele		
T861C	DCM	78	18	138	5.33	<0.05
	control	100	4	196		
T1338C	DCM	78	16	140	4.18	<0.05
	control	100	6	194		
T1908C	DCM	78	58	98	11.52	<0.05
	control	100	30	170		

The table means T1908C, T861C and T1338C have different contribution in DCM and control. It shows C allele is significantly increased in DCM in 3 SNP sites.

increasing now. DCM should be differentiated from secondary causes of ventricular dilatation and dysfunction due to systemic or cardiac disease like ischemic heart disease, valvular heart disease, hypertensive cardiomyopathy, alcoholic cardiomyopathy, myocarditis, peripartum cardiomyopathy and doxorubicin toxicity. However, in these cases of "secondary" DCM, a genetic predisposition for development of DCM could be present either after, due to a single gene or polygenic factors. Lamin A/C is a nuclear intermediate filament that is one of the major structural components of the lamina network underlying and mechanically supporting the nuclear envelope. Lamin A/C probably also has a role in cell division, nuclear growth, and the anchorage of nuclear envelope proteins [8]. Lamin A/C has three different parts: a central, a-helical, coiled-coil rod domain, a non-helical N-terminal head, and a C-terminal tail. Most of the mutations associated with cardiac abnormalities are located in the central rod domain. Defects in the lamin A/C gene have been shown to be responsible for different diseases, Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy type 1B, Dunnigan-type familial partial lipodystrophy, Charcot-Marie-Tooth disease, mandibuloacral dysplasia, Hutchison-Gilford progeria, multisystem dystrophy syndrome, and DCM with conduction abnormalities [9]. The lamin A/C gene is a promising candidate gene for DCM and disease-causing mutations have been reported in previous studies [9].

Lamin A/C deficient (LMNA-/-) mice were reported it develop rapidly progressive DCM characterized by left ventricular (LV) dilation and reduced systolic contraction [10]. Isolated LMNA-/- myocytes show reduced shortening with normal baseline and peak amplitude of Ca^{2+} transients. LMNA-/-LV myocyte nuclei have marked alterations of shape and size with central displacement and fragmentation of heterochromatin; these changes are present but less severe in left atrial nuclei [10]. Also electron microscopy of LMNA-/- cardiomyocytes shows disorganization and detachment of desmin filaments from the nuclear surface with progressive disruption of the cytoskeletal desmin network [10].

Although LMNA several gene mutation sites were found in DCM patients, such as N195K and R225X [11]. A severe phenotype in N195K mutation carriers and preferential cardiac conduction disease in R225X carriers was encountered [11]. There are very few reports of LAMN mutation in Chinese Han race DCM patients. So we screened for the LAMN gene in Han patients with DCM. Our results show that there are no missense mutations in all samples. There are several reasons for our results. First, there are differences in the phenotypes of patients evaluated in the different studies. Or LMNA gene related DCM exhibits very serious phenotype just as complete AV block and the patients die very early after born. Second, frequency of LAMN mutation is very little in Han race DCM patients, so the number of cases and spectrum of heart defects studied were not more enough. Finally, LAMN gene mutations may not be existed in patients with DCM after born.

However we have found 3 nonsense single nucleotide polymorphisms (SNP) have statistical distribution difference in DCM samples and controls, which include T1908C, T861C and T1338C. LMNA gene T1908C is the silent mutation which is located in the end of exon 10 [12]. So T1908C selectively splicing transcripts decide lamin A and lamin C relatively content [13], which may affect the relative content of mRNA and protein production, thus affecting the gene function. However the mechanism of T861C and T1338C in DCM is still unclear. So our observations suggest that LMNA gene mutation is rarely found in DCM in Chinese Han

Race children and the SNP of LMNA gene may be associated with the susceptivity of DCM in Chinese Han children.

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Disclosure of conflict of interest

None.

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