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Original Article

Mutation in δ-Sg Gene in Familial Dilated Cardiomyopathy

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Abstract

Background: Mutations in different genes including dystrophin-associated glycoprotein complex caused familial dilated cardiomyopathy which is a genetically heterogeneous disease. The δ-SG gene contains nine exons spanning a 433-kb region of genomic DNA. It encodes a 35-kDa, singlepass, and type II transmembrane glycoprotein. **Materials and Methods:** In this study for the first time in Iran we screened 6 patients of a large family that they had positive family history of MI or sudden death by next generation sequencing method. **Results:** By employing NGS method we found missense mutation (p.R97Q) of δ-SG gene in 2 of 6 patients. **Conclusions:** The missense mutation (p.R97Q) in familial DCM patients is reported for the first time in Iranian patients with cardiac disease. Although this mutation is already known in other populations in Iran, it is not reported before.

Keywords: *Familial dilated cardiomyopathy, genetic analysis, next generation sequencing, δ-SG gene*

Introduction

Familial dilated cardiomyopathy (DCM) is a genetically heterogeneous disease caused by mutations in many different genes including those encoding proteins of the dystrophin-associated glycoprotein complex (DGC).[1,2] In cardiac and skeletal muscle, the DGC complex is primarily composed of the four components α-, β-, γ- and δ-sarcoglycan, essentially involved in sarcolemmal integrity by interaction with integrins that mediates cell adhesion to the extracellular matrix[1,2] and the cytoskeleton, thus protecting muscle fibers from contraction-induced damage and necrosis.[3,4] Mutations in these genes may cause disease phenotypes that range from DCM to muscular dystrophy.[1] This disorder has an incidence of 3.5–8.5/100,000 population per year and a prevalence of approximately 36/100,000 population, which appears to be on the rise.[5-7] The δ-SG gene contains nine exons spanning a 433-kb region of genomic DNA. It encodes a 35-kDa, singlepass, type II transmembrane glycoprotein.[8] The role of SGCD mutation in DCM disease is still under investigation and new aspects are being discovered every day.

In this study, for the first time, we screened 6 patients of a large family that they had positive family history of MI or sudden death and we found mutations in 2 of them. The aim of this study is screening of one family with history of several cardiac sudden death to recognize mutation and probably mutation carriers at risk.

Materials and Methods

Patient selection

A family (6 members) was referred the Chamran Cardiac Hospital of Isfahan University of Medical Sciences included in this study. All patients were evaluated by history, physical examination, Echocardiography and electrocardiography (ECG). After filling of informed consent, Blood samples (10 cc) were collected from each patient. Furthermore, 100 healthy people with no history of any cardiac disorder problems were selected as control group.

DNA extraction

Genomic DNA was isolated from peripheral blood lymphocytes using QIAamp DNA blood mini Kits (Qiagen, USA).

DNA quality check

DNA isolates were qualitatively and quantitatively analyzed using a Nanodrop Spectrophotometer. Agarose gel electrophoresis was carried out to check for

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RNA contamination or DNA degradation. All isolates were thus verified to be pure and of good quality.

Ion torrent personal genome machine (workflow)

This began with a customized Ampliseq panel obtained from www.ampliseq.com (LifeTech). Amplification panel consists of primer pairs that cover all exons-only of 77 selected genes with research evidence of causality linked to inherited cardiac conditions Ampliseq design resulted in a total of 3340 amplicons amplified in each sequencing run. Amplicons ranged from 67 to 191 bp lengths (median 160 bp). Library preparation was performed by target amplification using this Ampliseq panel of primers and the Ion Ampliseq Library Kit (Cat no. 4475345, California, USA), resulting in a single sample library. In our experiments, 10 patient samples were multiplexed for a single Ion Torrent 318 Chip. Each sample library was assigned a barcode adapter to differentiate between samples. The 10 sample libraries were then normalized to a concentration of 100 pM using the Agilent Bioanalyzer 2100 before they were combined into a collective template for subsequent processing.

Template preparation was carried out using 2 systems, the Ion OneTouch system and Ion OneTouch Enrichment System. Using the Ion personal genome machine (PGM) OneTouch 2 200 Kit (Cat no. 4480974, California, USA), amplicons were clonally amplified on Ion Sphere Particles (ISPs) via emulsion polymerase chain reaction (PCR). Subsequently, enrichment was achieved using Ion PGM enrichment beads (Cat no. 4478525).

Upon initialization of the Ion PGM following manufacturer's instructions with the Ion PGM Sequencing Kit V2 reagents (Cat no. 4482006, California, USA), Template positive ISPs were individually loaded onto an Ion 318 chip (Cat no. 4484354, California, USA). Sequencing was thus performed.

Validation by target polymerase chain reaction and Sanger sequencing

As Sanger sequencing remains the gold-standard for sequence validation, it was used to validate all curated variants identified by PGM. Primers were designed by submitting each target reference sequence (Hg19) to Primer3Plus, online primer design software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). Primer pairs used for target PCR in this study are listed in Table 1.

Purified PCR-grade primers were ordered through Integrated DNA Technologies.

PCR was performed using HotStarTaq Plus Master Mix Kit (Cat no. 203645, California, USA): Hotstart MasterMix 25 μL, Nuclease-free water 16 μL, forward/reverse primers 2 × 1 μL DNA 2 μL, Coral Load 5 μL. Optimized cycle conditions: 95C 5 min, (95C 30 s, 56C 30 s, 72C 30 s) x32 cycle, 72C 5 min. PCR products were visualized on an agarose gel to confirm the presence of single product amplification. PCR products were purified using the Qiaquick PCR Purification kit (Cat no. 28106, California, USA) before submitting for Sanger sequencing (Axil Scientific, Singapore). Sanger sequencing results were aligned to reference Hg19 to identify mutations using the CLC Genomics Workbench 2 software (QIAGEN Company).

Results

We screened 6 patients of familial DCM family that had positive family history of sudden death in young ages. The clinical characteristics of these patients are shown in Table 2. Figures 1 and 2 show Sanger sequencing result and pedigree of family, respectively. Also, molecular characterization of these mutations is shown in Table 3.

Discussion

In our study for the first time we used next generation sequencing method to find gene mutation in familial DCM patient. By employing this method, we able to screening 77-cardiac related gene in population that so far there has been no information about molecular genetics state.

The patient II-2 was a 31-year-old male that his first presentation was 6 years ago with severe palpitation and chest pain and ventricular tachycardia. The baseline ECG of the patient showed poor R wave progression and T wave abnormal repolarization so that would be characteristic of DCM [Figure 2]. The second presentation was a true syncope followed by implantable cardiovarter defibrillator (ICD) implantation. The result showed a

Table 1: Primer pairs were used for amplify δ-SG gene

Gene	Primer pair sequences
SGCD	Forward: 5'-CAATTGGCGCCAGAACACAA
	Reverse: 5'-TGACATTCACTTGCCCGGAA

Table 2: Clinical characteristics of patients

n	Sex	Age	Age of onset of disease	Sudden death	Palpitation and chest pain	Mutation analysis of δ-SG
I-2	Male	55	55	At 55	Yes	-
II-7	Male	38	37	At 38	Yes	-
II-5	Male	41	39	At 41	Yes	-
II-3	Male	34	34	-	Yes	p.R97Q
II-2	Male	31	25	-	Yes	p.R97Q
II-4	Female	30	-	-	-	No mutation found
II-1	Male	29	-	-	-	No mutation found
III-1	Male	6	-	-	-	No mutation found
III-2	Male	2	-	-	-	No mutation found

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heterozygous p.R97 Q missense mutation in SGCD. Our patient shows malignant clinical presentation in the presence of this specific type of mutation which is in concordance with previous findings of DCM. Tsubata *et al*., 2000[5] for the first time reported this mutation in one case with DCM, a c. 290G®A transition in exon 4, which changes the arginine at codon 97 to glutamine (R97Q), was identified. Another DCM patient was found in the family (II-3). The genetic testing in this patient also showed p.R97Q missense mutation in SGCD but ECG of II-3 does not look characteristic of DCM and this patient is an asymptomatic mutation carrier. I-2 of pedigree was grandfather that died suddenly with congestive heart failure (CHF) at 55. Two sons of I-2 died suddenly with CHF at ages 38 years and 41 years (II-7, II-5). Alive brothers (II-3 and II-2) are positive mutation for δ-SG gene mutation. This result is similar to result of Tsubata *et al*.,[5] They identified a heterozygous 451T-G transversion in exon 6 of the δ-SG gene, resulting in a ser151-to-ala (S151A) substitution, in a family with DCM (CMD1 L; 606685) in 3 generations.[5] The grandfather died suddenly at the age of 38 years with CHF. Two daughters died suddenly with CHF at ages 14 years and 36 years. The second of these daughters had 2 sons, 1 of whom died suddenly at age 17 with CHF while the other underwent cardiac transplantation at the age of 21 years. The S151A mutation was not detected in 100 controls. In comparison, our result showed that we have no mutation and sudden death in third generation (III-1 and III-2), but the family worked by Tsubata *et al*., detected with some members' sudden death in third generation as well.

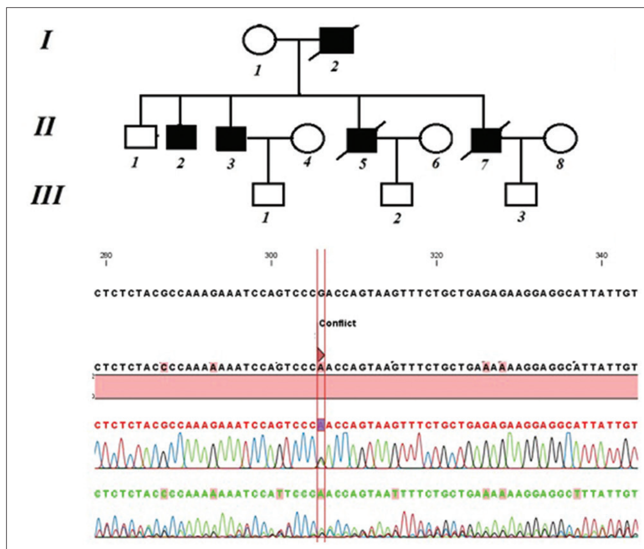

Figure 1: (a) Pedigree of family 1 and (b) Chromatogram of Sanger sequencing done to cover *SGCD2*, c. 290G > A, p.Arg97Glu. This mutation was found in 2 member (II-3, II-4) of family

Conclusions

The description of mutations in d-*sarcoglycan* in DCM patients provides more support for the concept that the final common DCM pathway is the cytoarchitecture which comprises the cytoskeleton, sarcolemma, and interacting components.[5] These results suggest that detection of DCM-associated mutations can improve presymptomatic diagnosis, enable better follow-up of asymptomatic patients and facilitate choosing effective therapies earlier. About this mutation is already known in other populations but it is not reported before in Iran; in a future study, we are planning to conduct the functional analysis of this mutation to characterize functional alterations in mutant protein.

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Nil.

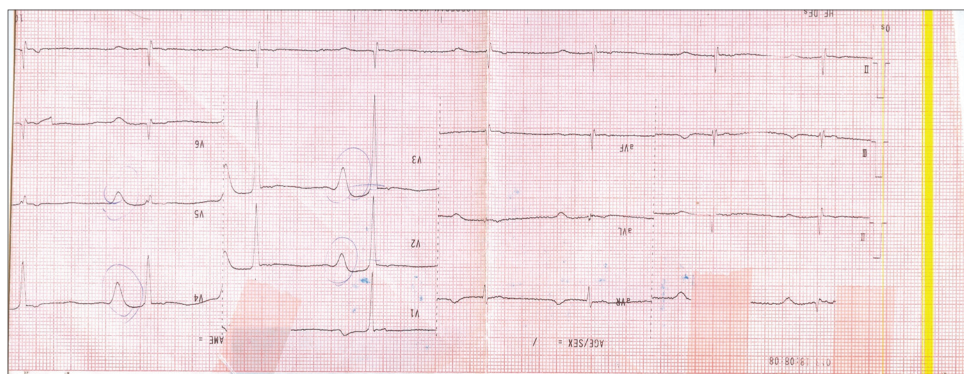

Figure 2: Electrocardiography of II-2 of pedigree

Table 3: Molecular characteristics of gene mutation finding

Patient ID	Gene name	Nucleotide change	Aminoacid change	Mutation type	Location	Phenotype (s)	Reference dbSNP
II-2, II-3	δ-SG	c. 290G>A (hetero)	p.Arg97Glu	Missense Deleterious	Extracellular domain of protein	Cardiomyopathy, dilated, 1L	rs45559835

dbSNP: Single nucleotide polymorphism database

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Conflicts of interest

There are no conflicts of interest.

References

1. Rutschow D, Bauer R, Göhringer C, Bekeredjian R, Schinkel S, Straub V, *et al.* S151A d-sarcoglycan mutation causes a mild phenotype of cardiomyopathy in mice. Eur J Hum Genet 2014;22:119-25.
2. Yoshida T, Pan Y, Hanada H, Iwata Y, Shigekawa M. Bidirectional signaling between sarcoglycans and the integrin adhesion system in cultured L6 myocytes. J Biol Chem 1998;273:1583-90.
3. Goehringer C, Rutschow D, Bauer R, Schinkel S, Weichenhan D, Bekeredjian R, *et al*. Prevention of cardiomyopathy in d-sarcoglycan knockout mice after systemic transfer of targeted adenoassociated viral vectors. Cardiovasc Res 2009;82:404-10.
4. Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 1992;355:696-702.
5. Tsubata S, Bowles KR, Vatta M, Zintz C, Titus J, Muhonen L, *et al.* Mutations in the human delta-sarcoglycan gene in familial and sporadic dilated cardiomyopathy. J Clin Invest 2000;106:655-62.
6. Manolio TA, Baughman KL, Rodeheffer R, Pearson TA, Bristow JD, Michels VV, *et al.* Prevalence and etiology of idiopathic dilated cardiomyopathy (summary of a National Heart, Lung, and Blood Institute workshop). Am J Cardiol 1992;69:1458-66.
7. Codd MB, Sugrue DD, Gersh BJ, Melton LJ 3rd. Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975-1984. Circulation 1989;80:564-72.
8. Blain AM, Straub VW. δ-sarcoglycan-deficient muscular dystrophy: From discovery to therapeutic approaches. Skelet Muscle 2011;1:13-8.