Original Article

Genetic analysis of Iranian family with hereditary cardiac arrhythmias by next generation sequencing

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Abstract

Background: Cardiac arrhythmias are responsible for several cases of syncope and sudden cardiac death annually worldwide. Due to overlapping clinical symptoms in some cardiac arrhythmias genetic studies would help to confirm the primary clinical diagnosis made on the basis of solely clinical findings. In addition clinical management of the patient, family screening and provide appropriate counseling and risk assessment for the family members are other advantages of genetic study.

Materials and Methods: Totally nine patients from a family included in this study. The primary diagnosis on the basis of clinical findings was second-degree atrioventricular (AV) block for this family. Mutation in *SCN5A* gene is frequently reported for second-degree AV block and hence the gene was analyzed using whole gene sequencing but no mutation was detected. Subsequently, the samples were subjected to customized Ampliseq 77 gene panel using next generation sequencing to detect the underlying molecular defects.

Results: We found *c. 5570T>A* missense mutation in *ANK2* gene for this family. Based on the Online Mendelian Inheritance in Man, *ANK2* gene and the mutation detected correspond to long QT syndrome type 4.

Conclusion: This mutation, although already known in other populations, but is reported for the first time in Iranian patients with cardiac arrhythmias. As the case with this family, genetic analysis of patients with cardiac arrhythmias would be helpful in reassessment of clinical diagnosis and therefore would help for patients' management and in some cases re-evaluation of ongoing treatment may be needed.

Key Words: Atrioventricular block, congenital long QT syndrome, hereditary cardiac arrhythmias, next generation sequencing

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INTRODUCTION

Cardiac arrhythmias are responsible for several million cases of syncope and sudden cardiac death (SCD) every year throughout the world. Primary familial or hereditary arrhythmias comprise a significant percentage of all forms of

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arrhythmias.[1-7] Genetic defects have been described in various forms of hereditary arrhythmias, e.g., long QT syndrome (LQTS), Brugada syndrome, short QT syndrome, sick sinus syndrome, cardiac conduction defect, catecholaminergic polymorphic ventricular tachycardia (CPVT), atrial fibrillation etc.[1-7] Patients with any one of these disorders usually have a normal heart (no structural abnormality, or coronary artery disease), but could suffer from cardiac arrhythmias, syncope and even SCD. Germ line or somatic mutations in different cardiac ion channel genes are hold responsible for the development of cardiac arrhythmias.[1-7] In many countries, regular genetic investigations for cardiac arrhythmias are now in routine clinical practice. Knowledge of underlying genetic defects would help in confirming the clinical diagnosis, useful in clinical management and providing appropriate genetic counseling for the family with an affected member.[1-7] Finding the causal gene and mutation would also help in efficient management of arrhythmia, one such example is making a decision between flecainide treatment or surgical sympathetic denervation in CPVT patients with a positive mutation in RyR2 gene.[8,9]

Genetic subtypes of inherited cardiac conditions (ICCs), such as LQTS, are associated with distinct patterns of risk and a molecular diagnosis can be used to direct clinical management and permit cascade screening of the family members, which is more effective than clinical screening alone.[10] However, ICCs are genetically heterogeneous, and conventional sequencing of ICC genes is expensive, time-consuming and rarely and inadequately applied in clinical practice, notwithstanding published guidelines.[10]

The maturation of recently developed next-generation sequencing (NGS) technologies provide unprecedented sequencing capacity at dramatically lower cost, fast and more scalable than other sequencing techniques.[10]

Since no mutation detected in *SCN5A* gene for this family, we performed NGS in order to identifying the causative genetic defect of cardiac arrhythmia for this family.

MATERIALS AND METHODS

Materials

All patients included in this study were recruited from the study of patients' hospital records at Shahid Chamran Hospital, Isfahan, Iran during 2012–2013. Proper informed written consent was obtained from all participants. As per their medical records, patients were diagnosed with A.V. Block.

DNA extraction and *SCN5A* gene analysis

Genomic DNA was isolated from peripheral blood lymphocytes using QIAamp DNA blood mini Kits (Qiagen, USA). The isolated DNAs were qualitatively and quantitatively analyzed using gel electrophoresis and Nanodrop Spectrophotometer, respectively. All exons and exon-intron boundaries of *SCN5A* gene were polymerase chain reaction (PCR)-amplified separately and subjected to Sanger sequencing.

We used customized Ampliseq panel obtained from www.ampliseq.com (LifeTech) for the genetic screening of this family. Amplification panel was consisted of primer pairs that cover all exons-only of 77 selected genes with research evidence of causality linked to ICCs. 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), resulting in a single sample library. In our experiments, three patient samples were multiplexed for a single ion Torrent 318 Chip. Each sample library was assigned a barcode adapter to differentiate between samples. The three 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 two systems, the Ion OneTouch system, and Ion OneTouch Enrichment System. Using the ion PGM OneTouch 2 200 Kit (Cat no. 4480974), amplicons were clonally amplified on ion sphere particles (ISPs) via emulsion 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), Template positive ISPs were individually loaded onto an ion 318 chip (Cat no. 4484354). Sequencing was thus performed.

Validation of detected mutations by Sanger sequencing

Sanger sequencing used to confirm the variants identified by PGM. Primer pairs for *ANK2* gene were designed using Primer3 Plus software. PCR was performed using HotStarTaq *Plus* kit (Cat no. 203645) using the following primer pairs: Upstream primer AGTCAAAGGCAAGGAGGACG and downstream primer GTGGGGGATACAGGTGGTTG. Optimized

thermal cycle condition used was: 95°C 5 min, (95°C 30 s, 56°C 30 s, 72°C 30 s) × 32 cycles, and a cycle of final extension at 72°C for 5 min. PCR products were visualized on an agarose gel to confirm the presence of single product amplification. Sanger sequencing results were aligned to reference normal gene sequence in order to identify the mutations.

RESULTS

We screened nine patients in this family. Clinical diagnosis made for this family on the basis of clinical findings was second-degree atrioventricular (AV) block. *SCN5A* gene sequence analysis revealed no mutation for members of this family. Subsequent NGS analysis using a customized Ampliseq panel of 77 cardiac related genes showed *ANK2, c. 5570T>A*, p.Val1857Glu mutation. Clinical characteristics of the patients are shown in Table 1. Sanger sequencing result and pedigree of the family are shown in Figure 1. Furthermore, molecular characterization of this mutation is shown in Table 2.

DISCUSSION

Here we report the genetic analysis performed for an Iranian family with hereditary cardiac arrhythmia. The clinical diagnosis of this family was AV block. The responsible gene for this cardiac arrhythmia is *SCN5A*. In order to find out the mutation, all exons, and exon/intron junctions of *SCN5A* gene were subjected to direct sequencing but no mutation was detected. Subsequently DNA samples were analyzed using 77-cardiac related array. Detected mutation in *ANK2* gene (*c. 5570T>A, p.Val1857Glu*), was related to LQTS. LQTS is caused by mutations in genes encoding ion channels controlling ventricular repolarization, or mutations in proteins that affect the function of these channels. Patients with LQTS may present with syncope, seizures, or sudden death.[11] This disease is genetically heterogeneous. Until date, 13 genes have been linked to LQTS, but more than 90% of genetically defined LQTS patients have a disease-causing mutation in any one of *KCNQ1*, LQT1, Online Mendelian Inheritance in Man (OMIM#) 607542, *KCNH2*, LQT2, OMIM# 152427, *SCN5A*, LQT3, OMIM# 600163, *ANK2*, LQT4, OMIM#600919, *KCNE1*, LQT5, OMIM#613695, and *KCNE2*, LQT6, OMIM#613693 genes.[12]

The clinical picture of LQTS is varies greatly: The patient can be asymptomatic or show recurrent syncope and seizures, or sudden death as the first manifestation of the disease. The presentation of the disease is mainly monogenic, polygenic or composite varieties usually have a more severe phenotype. Penetrance of disease, patients who have the mutation and manifest the phenotype, ranges from 25% to 90%. Less frequently, there may be variations in the expressivity of the disease, with several phenotypes resulting from the same mutation.[13]

In this family, we identified missense p.Val1857Glu in the repeated region (regulatory domain) of *ANK2* gene that causes loss of function mutation. This mutation was previously recorded in db SNP with SNP ID of rs141212932 and under Ensembl with ENSE00001003852.

*ANK*2 encodes the adapter protein of 220-kDa named ankyrin-B, which is critical for targeting ion

Table 1: Clinical characteristics of the patients studied

Patient ID	Type of arrhythmia	Sex	Age (years)	Age of arrhythmia onset (years)	ECG	Family history		Genetic testing result	Progression of disease and treatment
					AV block	Syncope	SCD <30 years		
II-1	AV block	Male	50	25	Normal	Yes	No	ANK2	Severe palpitation and ICD[a] implantation
II-2		Female	45	22	Normal	Yes	No	ANK2	Severe palpitation and ICD implantation
II-3		Female	40	19	Normal	Yes	No	ANK2	-
III-5		Female	38	24	Normal	No	No	ANK2	Severe palpitation and ICD implantation
II-4	Unaffected	Female	39	-	Normal	No	No	Negative	-
II-6		Male	34	-	Normal	No	No	Negative	-
III-1		Female	16	-	Normal	No	No	Negative	-
III-2		Female	13	-	Normal	No	No	Negative	-
III-3		Male	9	-	Normal	No	No	Negative	-

[a]ICD: Implantable cardioverter-defibrillator. ANK2: Ankyrin 2, SCD: Sudden cardiac death, AV: Atrioventricular, ECG: Electrocardiography

Table 2: Molecular characteristics of detected mutation

Gene name	Nucleotide change	Amino acid change	Mutation type	Location	OMIM	Reference (dbSNP)
ANK2	c. 5570T >A	p.Val 1857Glu (hetero)	Missense	Repeat-rich region Exon 38	Long QT syndrome 4	rs 141212932

OMIM: Online Mendelian Inheritance in Man, ANK2: Ankyrin 2, dpSNP: Single nucleotide polymorphism database

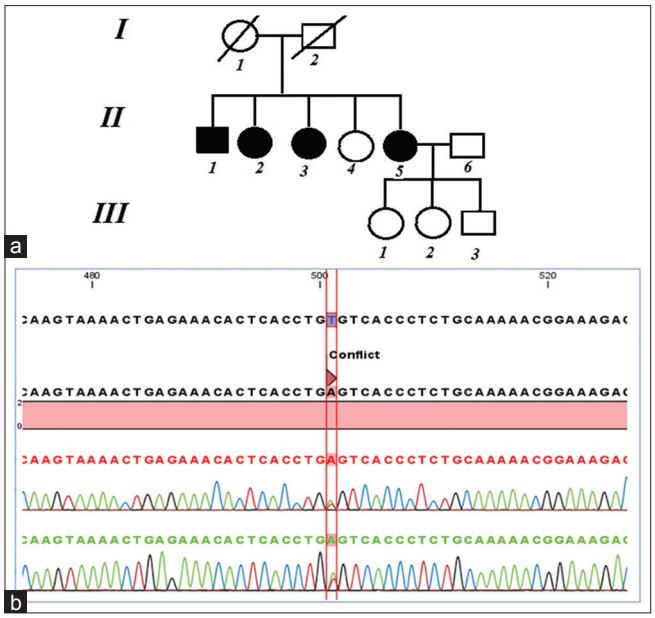

Figure 1: (a) Pedigree of the family and (b) chromatogram of Sanger sequencing done to cover *ANK2, c. 5570T>A, p.Val1857Glu.* This mutation was found in all four affected family members

channels and transporters to membrane domains in ventricular cardiomyocytes. Specifically, ankyrin-B directly associates with and targets Na/K ATPase and Na/Ca exchanger to cardiomyocyte transverse-tubule membranes. Additionally, inositol triphosphate receptors are targeted by ankyrin-B to the myocyte sarcoplasmic reticulum membrane.[14] Mutations in this gene cause long QT syndrome type 4 (LQT4). Humans with ankyrin-B mutations display varying degrees of cardiac dysfunction including bradycardia, sinus arrhythmia, idiopathic ventricular fibrillation, CPVT, and risk of sudden death.[15] Abnormalities of the heart's natural pacemaker (the sinoatrial node) and bradycardia are evident in almost all patients with *ANK2* mutation. Furthermore, coexistence of AV block (primary clinical diagnosis made for the family participated in our study) with LQTS4 has been observed before.[13]

Zhou, *et al*.[16] performed screening for *ANK2* gene mutations in 78 unrelated Japanese patients and reported a novel missense mutation in exon 40 of *ANK2* gene that responsible for LQT4. Several missense mutations including E1813K, R1788W, which have been identified in recent studies all occur in or near the regulatory and functional domains of exons 36–46 of the gene. Also, p.Val1857Glu mutation that we identified in this family is located in exon 38 and leading to loss of function of ankyrin-B protein.[17,18]

By employing customized Ampliseq 77 gene panel using NGS method quick cardiac arrhythmia related gene screening is possible. In this study, rapid analysis of a large cohort of related genes resulted in detection of a LQTS associated mutation. In this way, detection of asymptomatic mutation carriers, presymptomatic diagnosis, and more adequate personalized and earlier therapies are possible.[16]

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Conflicts of interest
There are no conflicts of interest.

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