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Development and diversity of a novel panel of short tandem repeat markers encompassing the *SCN5A* gene in Iranian population

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Introduction

The *SCN5A* gene plays a key role in a variety of heterogeneous cardiac diseases such as congenital long QT syndrome, Brugada syndrome and sudden cardiac death. The substantial utility of highly polymorphic short tandem repeat (STR) markers in forensic and diagnosis purposes prompted us to develop and validate a panel of six novel STR markers encompassing the *SCN5A* gene. Allele frequencies and forensic statistics of six tetranucleotide tandem repeat markers identified by tandem repeats finder (TRF) and SERV programs and amplified in a six-plex PCR system were calculated in 60 unrelated Iranian healthy individuals. Fragment analysis revealed 6–10 alleles in six STR markers with an observed heterozygosity greater than 0.667 in five markers. The power of discrimination was more than 0.83 for the panel. This novel panel of six polymorphic STR markers with high level of heterozygosity and discrimination in each locus can help to establish a rapid and more reliable identification of the disease causative *SCN5A* gene and provide aid to forensic purposes and prenatal diagnosis.

The *SCN5A* gene plays a key role in propagation of cardiac action potential and is responsible for rapid depolarization of inward sodium current in cardiomyocytes by encoding the α-subunit of the voltage-gated sodium channel, $Na_V1.5$ (Hamosh *et al.* 2005).

Gain or loss of function mutations in *SCN5A* gene lead to a variety of heterogeneous cardiac diseases (Zaklyazminskayaa and Dzemeshkevicha 2016) such as congenital long QT syndrome (LQTS) in which the *SCN5A* gene is the third major gene among 16 genes accounting for the genetic-positive LQTS (Tester and Ackerman 2014; Reed *et al.* 2015). Brugada syndrome (BrS) is another arrhythmic disorder in which 18 causative genes were reported and the percentage of *SCN5A* gene variants was attributed to 11–28% (Sieira *et al.* 2016).

SCN5A is also included in the most commonly mutated genes, in dilated cardiomyopathy (DCM) with over 60 linked genes (Haas *et al.* 2014). Autopsy negative sudden cardiac death accompanied by no previous manifestations is resulted from the *SCN5A* gene mutations as a major component (Tester *et al.* 2012; Kauferstein *et al.* 2013; Hertz *et al.* 2015). Atrial fibrillation (Darbar *et al.* 2008), sick sinus syndrome (Benson *et al.* 2003) and idiopathic ventricular fibrillation (Chen *et al.* 1998) are some of the other overlapping syndromes associated with *SCN5A* gene mutations.

SCN5A consists of 27 coding exons spanning $\sim$ 80 kb (Hamosh *et al.* 2005). More than 500 pathogenic and likely pathogenic variants of the cardiac sodium channel gene have been submitted to ClinVar (Landrum *et al.* 2016). Therefore, diagnosis of mutated gene for such genetic heterogeneous diseases is time-consuming, unprofitable and additionally requires much DNA sample.

Haplotype analysis with short tandem repeat (STR) markers flanking the *SCN5A* gene can help to overcome the limitations. Further, the utility of highly polymorphic

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markers for forensic purposes, homozygosity mapping, the prenatal genetic diagnosis (PND) and preimplantation genetic diagnosis (PGD) approaches are previously advocated (Butler 2007; Zupanič Pajnič *et al.* 2010).

In the current study, we identified six novel tetranucleotide STR markers amplifiable in one multiplex-PCR reaction to rapidly identify the disease causative *SCN5A* gene in a family represented with the above cardiac diseases. We also presented the heterozygosity and frequency assessments of these STR markers in Iranian population.

Materials and methods

DNA preparation

Blood samples were collected from 60 unrelated healthy individuals of Iranian population. Informed consents were obtained from all participants. This study was approved by the ethics committees of Pasteur Institute of Iran (adopted from the 1975 Helsinki Declaration). Genomic DNA was isolated from peripheral blood according to the standard salting out protocol (Miller *et al.* 1988).

Markers identification

Five tetranucleotide tandem repeat markers flanking the *SCN5A* gene and one located inside the gene (figure 1) were identified and selected by UCSC genome browser and TRF (Benson 1999) and sequence-based estimation of repeat variability (SERV) programs (Legendre *et al.* 2007).

Markers amplification and visualization

Six primer pairs were designed and fluorescently labelled with either FAM, VIC or PET dyes to amplify these STR markers in one multiplex-PCR reaction (table 1). PCR amplification was performed in a 17 μL reaction volume containing 2.8 μL 10× Buffer, 3.94 mM $MgCl_2$, 3.2 mM dNTP, 1 μL bovin serum albumin, 5 U per μL KBC Taqplus DNA polymerase in addition to about 200 ng of genomic DNA. Thirty-five amplification cycles were carried out in 95°C for 5 min, 95°C for 1 min, 63°C for 1 min and 30 s, 70°C for 2 min, 70°C for 17 min. The fragments were separated on ABI 3130 Genetic Analyzer and GS500 LIZ was used as size standard. Data were analysed by GeneMapper ID software.

Statistical assessments

The allelic frequencies, observed and expected heterozygosity, probability of identity (PI) and the power of exclusion (PE) were calculated by the utility of GenAlEx 6.502 software. The power of discrimination (PD) for each locus was calculated by direct counting method using the formula PD = 1–PI. The deviation from Hardy–Weinberg equilibrium (HWE) along with the polymorphism information content (PIC) was evaluated by Cervus 3.0.7 program.

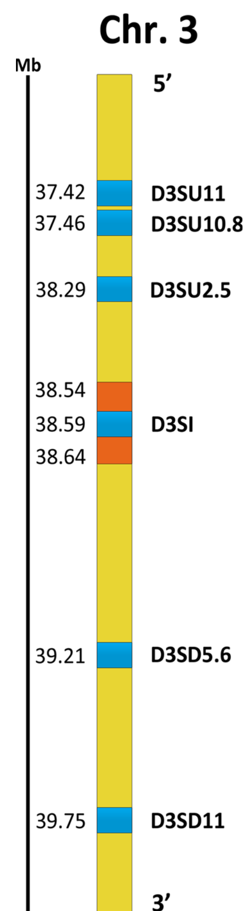

Figure 1. Relative positions of six STR loci encompassing the *SCN5A* gene on chromosome 3. The *SCN5A* gene (orange colour) is located on the short region of chromosome 3 from 38.54 to 38.64 Mb and D3SI (blue colour) is an intragenic marker. The numbers in the markers names show the distance between the gene and the flanking marker divided by 10^5 (i.e. 11 in D3SU11 means a distance of 1,100,000 bp from the gene), in the markers names 'U' stand for upstream of the gene; 'I', inside the gene; 'D', downstream of the gene.

Results and discussion

All the previously reported STR markers for the *SCN5A* gene were dinucleotide repeats but we used TRF and SERV programs for identification of tetranucleotide tandem repeats to eliminate the minor bands and to reduce the stutter bands during PCR amplification (Butler 2007; Rabbani *et al.* 2008). The maximum interval of 1.1 Mb around the *SCN5A* gene was specified to the tetranucleotide STR markers selection to minimize the meiosis recombination occurrence.

Table 1. Six STR markers linked to the *SCN5A* gene.

Marker	Consensus sequence	Repeat structure	PCR primer sequences	Dye label	Size range
D3SU11	GATA	$[GATA]_2[GAC]_1[GATA]_{12}[G]_1$	5′- TGACTTTGGCTTTACGGAGGTAG -3′ 5′- GCATGTCCTTAAGGAACTTGAGTTA -3′	VIC	299–318
D3SU10.8	AAAT	$[AAAT]_{12}[AA]_1$	5′- AGCAGTGCAGCAAGAATTTCTC -3′ 5′- CAATAGATAACTGAAACACTTGGCAT -3′	PET	134–161
D3SU2.5	ATCT	$[ATCT]_{12}[AATCT]_1[ATCT]_1[ATC]_1$	5′- TGGACAGAGGTAGAAAATAGACTTGC -3′ 5′- CCACAGTCTTCATTCTAGTCTTCCC -3′	VIC	143–167
D3SI	ATCC	$[ATCC]_2[ATCT]_1$ $[GTCC]_1[ATCC]_9[CTCC]_1[ATCC]_1$ $[TTCC]_1[CTCC]_1[TTCT]_1[ATCC]_1[AACC]_1$ $[ATCC]2[A]_1$	5′- GCTCTGCCTGATTTACTTACTACACC -3′ 5′- CTCCACCTACAAATTACAAAATCTCAA -3′	PET	220–236
D3SD5.6	AAAG	$[AAAG]_{18}[AAG]_1[AAAG]_3[AAA]_1[AAAG]_{12}$	5′- ACAAATTTGGAGCTGATTCTTAACTG -3′ 5′- CATCAGAAAGAGAACTAGGAGGAATCT -3′	FAM	239–281
D3SD11	CTAT	$[CTAT]_{11}$	5′- TTATCTTCTTTAAGACAATAGTGCCATG -3′ 5′- CCAGATAGCCGTTAGTATGTTAGATAATG -3′	PET	334–357

Table 2. Population data of six novel STR markers.

	D3SU11		D3SU10.8		D3SU2.5		D3SI		D3SD5.6		D3SD11	
Allele number	Allele size	Frequency	Allele size	Frequency	Allele size	Frequency	Allele size	Frequency	Allele size	Frequency	Allele size	Frequency
1	299	0.033	134	0.042	143	0.008	220	0.1	239	0.008	334	0.008
2	303	0.325	140	0.458	147	0.017	224	0.258	247	0.083	337	0.158
3	307	0.358	145	0.042	151	0.117	228	0.333	252	0.167	341	0.467
4	310	0.167	149	0.017	155	0.5	232	0.283	256	0.133	345	0.275
5	314	0.083	153	0.2	159	0.267	236	0.025	260	0.167	348	0.008
6	318	0.033	157	0.225	163	0.083			264	0.225	349	0.067
7			161	0.017	167	0.008			269	0.125	353	0.008
8									273	0.067	357	0.008
9									277	0.017		
10									281	0.008		
Na	6		7		7		5		10		8	
He	0.729		0.695		0.658		0.731		0.849		0.677	
Ho	0.75		0.433		0.667		0.683		0.917		0.683	
PI	0.12		0.14		0.17		0.12		0.04		0.16	
PD	0.88		0.86		0.83		0.88		0.96		0.84	
PIC	0.684		0.651		0.609		0.682		0.83		0.626	
PE	0.678		0.647		0.599		0.66		0.864		0.612	
HWE expected P value	0.59		<0.0002		0.88		0.94		< 0.0002		0.86	

Fragment analysis of six novel STR markers revealed 6, 7, 7, 5, 10 and 8 alleles for D3SU11, D3SU10.8, D3SU2.5, D3SI, D3SD5.6 and D3SD11, respectively. Allele frequencies and the Iranian population data for STR markers are shown in table 2. The mean number of alleles over all loci was 7.72. Five of six loci had the observed heterozygosity (H_o) greater than 0.667. The highest H_o was 0.917 at D3SD5.6 and the lowest was 0.433 at D3SU10.8 marker. The PD for all loci was more than 0.83. Despite the great extent of consanguineous marriages in Iranian population, the mean number of PIC for the panel was 0.68 which indicated that all loci were highly informative. There were no significant deviations from HWE for loci examined within the Iranian population except for D3SU10.8 and D3SD5.6. These two markers displayed the probability of less than 0.0002 when the deviations from HWE were re-examined by GeneAlEx software. Such deviations can be explained either by probable genotyping and laboratory errors or by population stratification.

In conclusion, this novel panel of six polymorphic STR markers with the high level of heterozygosity and discrimination in each locus can help to establish a rapid and more reliable *SCN5A* gene identification in heterogeneous cardiovascular diseases through the haplotype analysis. The panel is further an efficient tool in forensic, prenatal diagnosis, preimplantation of genetic diagnosis and homozygosity mapping, especially in the autosomal recessive diseases and endogamous populations with the high rate of the consanguineous marriages.

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