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Analysis of the sarcomere protein gene mutation on cardiomyopathy – Mutations in the cardiac troponin I gene

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ABSTRACT

Developments in the molecular genetic studies of cardiomyopathy (CM) have led to discovery of a large number of mutations in the genes encoding the sarcomeric proteins. In this study, comprehensive screening of TNNI3 was performed in 36 consented autopsy cases diagnosed as CM, in order to evaluate the prevalence of gene mutations in sudden death caused by CM. In DCM cases, a new missense mutation Pro16Thr was detected. A single nucleotide polymorphism at −8 position of intron 3 (IVS 3 −8 T>A) was identified, which had a significant difference in allele frequency between DCM and control cases. From these results, it was indicated that this study contribute to genetic based diagnosis, risk stratification and prevention of sudden death caused by CM.

1. Introduction

Sudden cardiac death is one of the most common causes of death. Many of the causes of sudden death, especially in the young, are due to genetic heart disorders, both with structural and arrhythmogenic abnormalities [1]. In these sudden death cases molecular pathological techniques may help to determine the cause and even manner of death [2,3].

Cardiomyopathy (CM) is defined as a disease of the myocardium characterized by the presence of systolic or diastolic dysfunction or abnormal myocardial structure and is classified into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC) and unclassified cardiomyopathy [4].

HCM, the most common cause of sudden death in the young adults, is an autosomal dominant disease characterized by ventricular hypertrophy accompanied by myofibrillar disarrays [5]. Molecular genetic studies of familial HCM have demonstrated that about half of the patients have mutations in the genes encoding sarcomeric proteins [6]. Three genes account for most known mutations: myocardial β myosin heavy chain (MYH7), cardiac myosin binding protein C (MYBPC3) and cardiac troponin T (TNNT2) [5]. The frequencies of these causative gene abnormalities in familial HCM are regarded as 19%, 11% and 12% for MYH7, MYBPC3 and TNNT2, respectively [7].

Thin filament associated regulatory proteins, troponin and tropomyosin subunits, are responsible for regulating the contractile force of the cardiac myofibrils [8]. Troponin is a complex of three different proteins, troponin C (Ca^{2+} binding component), troponin I (inhibitory component) and troponin T (tropomyosin-binding component). Troponin I has been shown to be in relative proximity to actin in the relaxed state and an increase in free intracellular Ca^{2+} and binding of Ca^{2+} with troponin C triggers the contraction mechanism [9]. The troponin I gene (TNNI3) is a 5966 bp gene located on chromosome 19 consisting of eight exons and encodes a protein of 210 amino acids [10]. Cardiac troponin I has considerable homology to the skeletal muscle isoform. The major difference between cardiac and skeletal troponin I is that cardiac troponin I contains a 30-residue N-terminal extension. This N-terminal region contains two adjacent serine residues which are phosphorylated by cAMP-dependent protein kinase (PKA) [11]. The most important functional sequence of TNNI3 is a short central stretch of residues referred to as the inhibitory region and the minimum inhibitory sequence consists of residues 137–148 [12]. Specific missense mutations of TNNI3 have also been found to cause familial HCM. Five HCM causing missense mutations (Arg145Gly, Arg145Gln, Arg162Trp, Gly203Ser and Lys206Gln) were first reported along with a mutation causing deletion of one codon (Lys^{183}) [13]. Thirty-five mutations and six gene polymorphisms have already been reported until now [http://genepath.med.harvard.edu/~seidman/cg3/muts/TNNI3_mutations_TOC.html]. Most of HCM causing mutations are found in Exon 7 and 8.

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Elucidating gene abnormalities is an important step towards curing the disorder, and it will be possible to predict a patient's prognosis and provide feedback to the family members. However, the prevalence of disease-causing genes in sudden death diagnosed as CM is not well defined. In this study, comprehensive screening of TNNI3 was performed in consented autopsy cases diagnosed as CM, in order to evaluate the prevalence of gene mutations in sudden death caused by CM.

2. Materials and methods

2.1. DNA sample

Blood samples were obtained from 36 consented autopsy cases diagnosed as CM, 15 cases of HCM, 18 cases of DCM and 3 cases of ARVC, with informed consent of their families members. Profiles of these subjects are shown in Table 1. ARVC is characterized by progressive fibrofatty replacement of right ventricular myocardium, initially with typical regional and later global right and some left ventricular involvement, with relative sparing of the septum [14]. Three cases diagnosed as ARVC in this study had morphologic evidence of the ARVC phenotype with fibrofatty replacement of right ventricular myocardium and dilatation of the right ventricle. Two hundred-twenty preserved samples that presented with no particular diseases were used as controls. DNA was extracted using Quick Gene-800 (FUJIFILM, Tokyo) and stored at 4 °C until use.

2.2. PCR and electrophoresis conditions

Primers were prepared for TNNI3 exons 1–8 (Table 2). DNA samples were amplified by PCR as follows. Amplification was carried out using 1.25 μl of 5–10 ng DNA as template and adding 1.25 μl STR 10 × Buffer, 0.05 μl Taq DNA Polymerase, 0.5 μl 10 μM Forward Primer, 0.5 μl 10 μM Reverse Primer, and adjusting the total volume to 12.5 μl with sterile deionized water. GeneAmp PCR system 9700 (Applied Biosystems) was used with an initial step of 94 °C for 3 min, then 30 cycles of denaturation at 94 °C for 45 s, annealing at 58 °C for 45 s, and extension at 72 °C for 45 s, with a final step of 72 °C for 5 min. PCR products were sequenced by direct sequencing using the ABI PRISM BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). After purification using BigDye® XTerminator™ purification kit (Applied Biosystems), electrophoresis was carried out for 36 min with POP6 polymer at 15 kV, 50 °C, and a capillary length of 47 cm using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with 310 Data collection Software Ver. 3.1. Sequencing data were analyzed by running SeqScape® Software Ver. 2.5.0. The resulting sequence data were compared with the reference sequence available on the NCBI database (NG_007866).

Table 2
PCR primers used for analysis of the TNNI3 exons.

Exon	Forward (5′ – 3′)	Reverse (5′ – 3′)
1	CGTTATCTGGCATAGTGGGC	TTGGGGCATCACTCACCCAT
2	TCCCTTAGGAGACAGGACACAGC	TTACCGTACCGCACCCTCTGCTA
3	GGTCTTGGTGGTCATGGGGT	TGTACTCTGCCCCCAGGAAG
4	TAGACTCAGGGCTCAAGTTG	AAAGCCCCACCCATTCTCAA
5	CTGGTCTTTATCCTGAAGCC	ATTCCGGGACTAGAAACCTC
6	CAACACACACCACGTTCCTC	AGAGACCAAGTCCCAGCCAT
7	GTAGGATGGAGGAGTTGGGTG	CCCCTCAGCATCCTCTTTCCT
8	AGAAGAGACCCTAACCTCTG	GAAGCTTTATTCCTCAGGGC

Table 1
Profiles of 36 sudden death cases diagnosed as cardiomyopathy.

No.	Sex	Age at sudden death (diagnosis)	Confirmed family history	Age at first presentation	Situation of sudden death
1	m	53(HCM)	No	na	Working
2	f	36(HCM)	No	na	na
3	f	58(HCM)	No	na	Exercising
4	m	49(HCM)	No	na	Sleeping
5	m	44(HCM)	No	na	Sleeping
6	m	64(HCM)	No	49	na
7	m	55(HCM)	No	na	Drinking
8	f	49(HCM)	No	na	Working
9	f	45(HCM)	No	na	na
10	m	26(HCM)	Yes	25	na
11	m	51(HCM)	No	31	Sleeping
12	m	63(HCM)	No	56	na
13	m	60(HCM)	No	na	Exercising
14	m	73(HCM)	No	63	Sleeping
15	m	53(HCM)	No	51	Sleeping
16	f	43(DCM)	No	23	na
17	m	61(DCM)	No	56	Bathing
18	f	43(DCM)	No	40	na
19	m	73(DCM)	No	49	Sleeping
20	f	31(DCM)	No	25	Exercising
21	m	76(DCM)	No	55	na
22	m	58(DCM)	No	48	Shopping
23	m	59(DCM)	No	54	Sleeping
24	m	32(DCM)	No	na	Exercising
25	m	54(DCM)	No	50	Working
26	m	57(DCM)	No	47	Sleeping
27	m	55(DCM)	No	52	na
28	f	88(DCM)	No	na	na
29	m	53(DCM)	No	51	na
30	m	69(DCM)	No	59	Sleeping
31	f	68(DCM)	No	59	Sleeping
32	f	77(DCM)	No	69	Sleeping
33	m	68(DCM)	No	66	na
34	m	33(ARVC)	No	30	Working
35	m	44(ARVC)	No	na	na
36	f	62(ARVC)	No	52	na

na: Not available.