Original article

Genetic analysis of the myocardial Na+ pump α subunit gene SCN5A mutation in 7 cases of sudden death among our 425 autopsy cases - gene mutation was suggested in 4 cases out of 7 ones by the polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) method.

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Introduction : Concerning the unknown sudden death in spite of the pathologic autopsy survey, we carried out the genetic study for cardiac sudden death.

Materials and methods : Among 7 cases of sudden death from 425 autopsied cases in our institution for recent 15 years, we examined myocardial Na+ pump α subunit gene SCN5A mutation with the DNA specimens derived from their formalin-fixed paraffin-embedded sections (FFPEs) by PCR-SSCP method.

Results : Genetic mutation was shown in 4 cases out of 7 ones, and they were correctly re-diagnosed as cardiac sudden death.

Conclusion : For the further investigation of the sudden death, the genetic analysis is effective.

Key words : genetic analysis, myocardial Na+ pump α subunit gene SCN5A mutation, cardiac sudden death, autopsy, gene mutation, polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) method

Introduction

Sudden death consisted of many causes : cerebral, cardiac, aortic, anaphylactic, drug-abuse and so on. The process of making final diagnosis has been improved by several new techniques : i.e. the autopsy imaging for a space-occupying hematoma, the immuno-histochemical study with anti-complement 9 antibody for an extremely early phase of myocardial necrosis (5, 7). A death cause of functional disturbances, like lethal arrhythmia and subsequent myocardial dysfunction, has been, nevertheless, left unsolved. Myocardial Na+ pump α subunit gene SCN 5A mutation has been identified as the cause of lethal arrhythmia (1, 6). Gene SCN5A consisted of 28 exons, 80 kb in size. Ito found that 33% of arrhythmic cases of Brugada syndrome revealed SCN5A mutation in exons 12, 18, or 20(6). We reviewed our unknown-sudden-death autopsied cases by SSCP analysis of these SCN5A mutations.

Materials and methods

DNA samples were extracted from FFPE specimens with neutral formalin fixative and DEXPAT (Takara) (Table 1) (4).

4 pairs of PCR primers were used against exons 12-1, 12-2, 18, and 20 of Na+ pump α subunit gene SCN 5A (Table 2) (6). PCR component and amplification setting were same as the previous one (Table 2) (3). The numbers of amplification cycles were from 30 to 120, depending on producing appropriate each single optimum electrophoretic band of PCR products. Mean PCR cycles were 30 for exon 12-1, 45 for exon 12-2, 80 for exon 18, and 120 for exon 20. Because of many amplification cycles, the chemical reagent adjustment manipulation like nested PCR was done during the amplification.

SSCP method with silver stain was complied with the previous report except several modifications : the use of half-sized electrophoresis device measuring 18 cm in length, and electrophoresis at 280V for 2 hours until blue-stained presentation markers flowed out (Table 2) (2).

The criteria judging the aberrant band as "positive" was as follows : 1. golden-colored bands similar as ones of the control case, 2. definite black bands, confirmed by the reexamination.

Results

Final PCR products were around 200-300bp.

Case 4 and 5 showed aberrant bands in exon 12-1 (Figure 1). Case 6 and 7 revealed extra bands in exon

18（Figure 1）. Cardiac sudden death was suggested in these four cases（Table 1）.

Discussion

Cardiac sudden death was mainly caused by myocardial infarct, which was confirmed by coronary obstruction and myocardial necrosis. Extremely early phase of myocardial infarct without typical myocardial necrosis became diagnosed by immune-histochemical positivity against complement 9（7）. But there seemed to be many cardiac sudden deaths arisen by lethal arrhythmia or myocardial dysfunction without any organic pathologic changes. Myocardial Na+ pump α subunit gene SCN5A mutation as the cause of lethal arrhythmia and myocardial dysfunction can support these sudden cardiac death（1, 6）. In this study four autopsied cases with unknown sudden death could be disclosed as cardiac sudden death based on SCN5A mutation. The aberrant stains in SCN5A-SSCP method were shown in 4/7（57%）, which was higher than that of the previous report（6）. These genetic analyses become effective in an accurate diagnosis of functional cardiac failure.

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和　文　抄　録

原　　著

ナトリウムポンプαサブユニット SCN5A 遺伝子変異を polymerase chain reaction-single strand conformational polymorphism（PCR-SSCP）手技で同定できた心臓性突然死の 4 症例

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目的：病理解剖診断における原因不明の突然死に関して、遺伝子検査による心臓性突然死を確定する。

方法：当施設で15年間に解剖された425症例中、病理診断が確定できなかった原因不明の突然死 7 症例に関して、ナトリウムポンプαサブユニット遺伝子 SCN5A 変異を検討する。ホルマリン固定パラフィン切片より回収した DNA 材料を使って、PCR-SSCP 検査を実施した。

成績：4 症例に遺伝子変異が示唆され、心臓性突然死と訂正診断できた。

結論：突然死の原因究明において、遺伝子検査は有効である。

キーワード：突然死、原因不明、解剖、心臓性突然死、遺伝子検査、心筋ナトリウムポンプαサブユニット SCN5A 遺伝子異常、polymerase chain reaction-single strand conformational polymorphism（PCR-SSCP）法

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Table 1. Summary of 7 cases in this study

#	autopsy #	y/o	sex	presentation	past history / present illness	addendum mutation
0	SN14-1	41	male	COL		control
1	SN14-2	82	male	DOA		
2	SN14-12	30	male	IP	Pn, Ath	
3	SN11-14	16	male	DOA		
4	SN08-2	81	male	DOA	AR	exon 12-1
5	SN08-5	85	female	DOA	Af, CI	exon 12-1
6	SN05-28	92	female	DOA	CEH, CI	exon 18
7	SN03-38	76	female	DOA	Af, HT, CI	exon 18

abbreviation

Af	atrial fibrillation
AR	arrhythmia
Ath	Asthma
CEH	chronic epidural hematoma
CI	cerebral infarct
COL	carcinoma of lung (cancer death)
DOA	death on arrival
HT	hypertension
IP	inpatient
Pn	pneumonia

Table 2. Operational procedure of PCR-SSCP

DNA preparation	DNA extraction, purification and concentration from paraffin-embedded sections with DEXPAT (Takara) following attached instructions
PCR primers	exon 12-1: gccAgtggctcAAAAgAcAggct cctgggcActggtccggcgcA exon 12-2: cAccAcAcAtcActgctggtgc ggAActgctgAtcAgtttgggAgA exon 18: AgggtctAAAcccccAgggtcA cccAgctggcttcAgggAcAAA exon 20: AcAggccctgAggtgggcctgA tgAcctgActttccAgctggAgA
PCR amplification	
contents	AmpliTaq Gold (Life Technologies)
	distilled water 14 μl
	10xPCR buffer 2 μl
	dNTP mix 2 μl
	primers 0.5 μl each
	DNA polymerase 0.2 μl
	DNA sample 2 μl
amplification	
1st step	hot start at 95°C, 10'
2nd step	denaturation at 93°C, 45"
	annealing at 53°C, 1'
	extension at 72°C, 10'
3rd step	last extension at 72°C, 10'
firtst round	exon 12-1: 30x at 2nd step exon 12-2: 30x at 2nd step exon 18: 40x at 2nd step exon 20: 60x at 2nd step
second round	exon 12-2: 15x at 2nd step exon 18: 40x at 2nd step exon 20: 60x at 2nd step
SSCP analysis	
PCR sample	PCR product 5 μl + dye buffer (99% formamide, 0.05% bromophenol blue, 0.05% xylol) 10 μl
single stranding denaturation	denaturation at 90°C, 10', in PCR thermal cycler
	cooling on alminium board at -20°C
electrophoresis	
polyacrylamide gel	12.5% polyacrylamide
	5% glycerol
buffer	1XTris borate EDTA (TBE) buffer
loading sample	15 μl
apparatus	slab (Nion eido, NA-1112), phoresis tank was attached with ice packs for cooling
method	280V, 2°
staining	Silver stain plus kit (BIO-RAD)

EDTA: ethylenediaminetetraacetic acid
PCR: polymerase chain reaction
SSCP: single strand conformation polymorphism

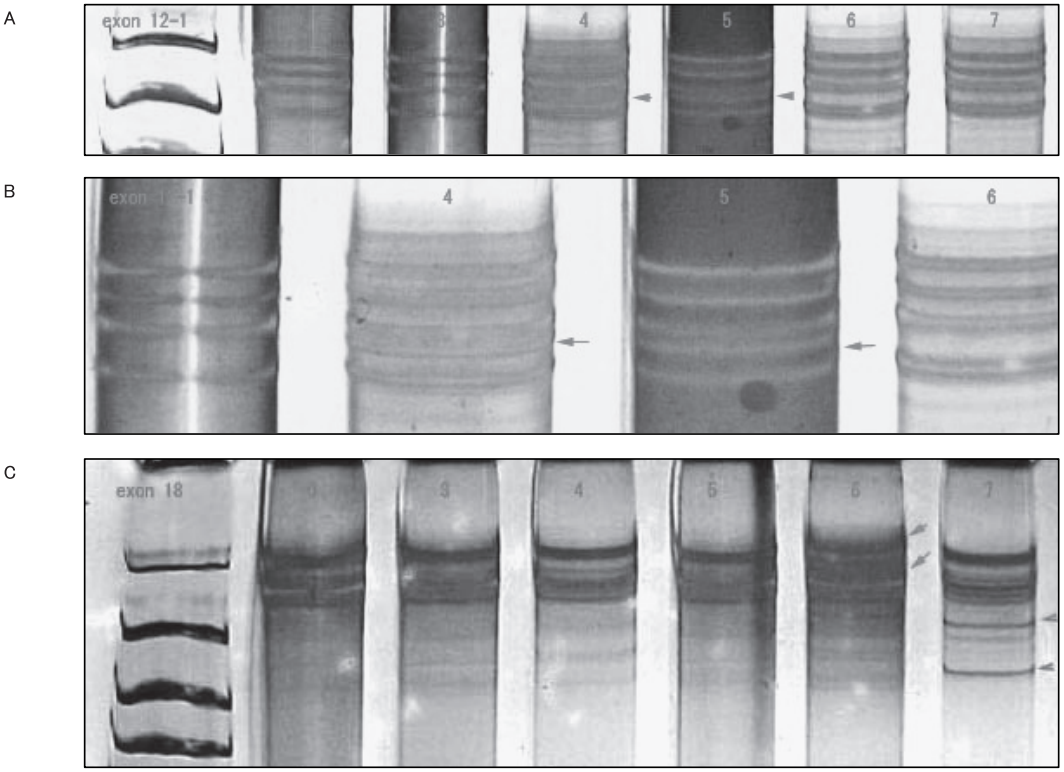

Figure 1. A : exon 12-1 PCR-SSCP, B : enlarged view of A, the third band from the top is duplicated in 4th and 5th cases, (arrow), extra band (arrow), C : exon 18 PCR-SSCP, dislocated bands are shown in 6th case(arrow), extra bands are found below the ordinary band cluster in 7th case(arrow). Left side lane is marker and the first lane (0) was control.

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