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Mutations of desmoglein-2 in sudden death from arrhythmogenic right ventricular cardiomyopathy and sudden unexplained death

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ABSTRACT

Desmoglein-2 (DSG2), a member of the desmosomal cadherin superfamily, has been linked to arrhythmogenic right ventricular cardiomyopathy (ARVC)which may cause life-threatening ventricular arrhythmias and sudden death. Fatal arrhythmias resulting in sudden death also occur in the absence of morphologic cardiac abnormalities at autopsy. We sequenced all 15 exons of DSG2 in DNA extracted from post-mortem heart tissues of 25 patients dying with ARVC and 25 from sudden unexplained death (SUD). The primers were designed using the Primer Express 3.0 software. Direct sequencing for both sense and antisense strands was performed with a BigDye Terminator DNA sequencing kit on a 3130 xl Genetic Analyzer. Mutation damage prediction was made using Mutation Taster, Polyphen and SIFT software. 2 DSG2 mutations (p. S1026Q fsX12, p. G678R)in two ARVC samples and 2 DSG2 mutations(p. E 896K, p. A858 V) in two SUD samples were identified, all the mutations were novel. We concluded that DSG2 mutations may not specific for ARVC and may be related to the fatal arrhythmic events even in patients with a morphological normal heart.

1. Introduction

The desmosome is a specialized junction that provides intercellular links in the desmosome–intermediate filament complex giving tensile strength to tissues that experience mechanical stress, such as the skin, myocardium and gastrointestinal mucosa [1]. It consists of five major component proteins encoded by three gene superfamilies: the desmosomal cadherins, desmoglein (DSG) and desmocollin (DSC); the armadillo family, plakoglobin (PG) and plakophilin (PKP); and the plakin family, desmoplakin (DP). DSC and DSG are the desmosomal adhesion molecules, DP links the desmosomal plaque to the IF cytoskeleton, and PG and PKP are adaptor proteins that link between the adhesion molecules and DP.

Recently, mutations in desmosomal genes have been linked to arrhythmogenic right ventricular cardiomyopathy (ARVC), [2–9] which is an inherited disease of the heart muscle characterized by replacement of myocyte by fibro-fatty tissue. ARVC may cause life-threatening ventricular arrhythmias and sudden death [10,11].

Cases of sudden, apparently natural, death in children and adults where no cause of death is found at autopsy may demonstrate morphologically normal myocardium. In this study, we use the term "sudden unexplained death SUD" while recognizing that it is a heterogeneous group of disorders and does not define a specific syndrome. SUD accounts for up to 30% of sudden death in adults, and is especially common in children, adolescents and younger adults.

In a previous study, we tested the plakophilin-2 (PKP2) mutation, which is the most common desmosomal mutation associated with ARVC, with heart tissue from patients dying of ARVC and SUD, and 24% ARVC patients and 24% SUD patients have PKP2 mutations [12], which suggests that PKP2 mutations maybe not specific for ARVC and may result in SUD. DSG2 has overlapping functions in binding PG and PKP2 and has been implicated in some families with ARVC. This current study is an extension of our previous study [12], and our goal now is to evaluate the mutations in DSG2 from the same set of patients.

2. Materials and methods

Study subjects. 25 cases of sudden cardiac death with the diagnosis of ARVC and 25 cases of sudden cardiac death(SCD) with

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the diagnosis of SUD were studied from a single medical examiner's office and consulted by a cardiovascular pathologist. The study was approved by the institutional review board. All cases were seen in consultation by the cardiovascular pathologist and forensic pathologist and examined in a similar fashion.

Genotyping and sequence alignment. Genomic DNA was extracted from postmortem heart tissue using standard techniques [13]. All coding exons and flanking intronic sequences of DSG2 (NM_001943.3) were amplified by polymerase chain reaction (PCR). Primers and PCR conditions are available on request. The primers were designed using the Primer Express 3.0 software. Direct sequencing for both sense and antisense strands was performed with a BigDye Terminator DNA sequencing kit on a 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Data was analyzed with Lasergene software for identification of mutations (DNASTAR, Madison, WI). A control group of 96healthy and unrelated subjects was used to exclude the possibility that detected mutations were common DNA polymorphisms. Mutation damage prediction was made using Mutation Taster software (http://www.mutationtaster.org), PolyPhen (http://genetics.bwh.harvard.edu/pph2) and SIFTS (http://sift.jcvi.org).

3. Results

3.1. Mutation analysis of PKP2 in ARVC samples

Two DSG2 mutations have been identified in 2 of 25 ARVC index cases (8%). Of the 2 DSG2 mutations, 1 was missense, 1 insertion-deletions mutation (Fig. 1). None of the detected nucleotide changes was found in 96 control samples.

The missense substitution was 2032G>A (G678R), which was novel, considered to be possibly damaging by PolyPhen, and to be benign by SIFT and Mutation Taster. The frameshift c.3075_3076insC(S1026Q fsX12) insertion was also novel. Details regarding the identified mutations are summarized in Table 1. The histologic finding of these two patients who have mutation were showed in Fig. 2.

3.2. Mutation analysis of PKP2 in SUD samples

Two DSG2 mutations have been identified in 2 of 25 SUD index cases (8%). Both of the 2 DSG2 mutations were missense mutation (Fig. 3). None of the detected nucleotide changes was found in 96 control samples.

One of the missense substitution was 2686G>A (E 896K), which was novel, considered to be possibly damaging by PolyPhen, and to be benign by SIFT and Mutation Taster. Another missense substitution was 2573C>T (A858V), which was also novel, considered to be benign by PolyPhen, SIFT and Mutation Taster. Details regarding the identified mutations are summarized in Table 1.

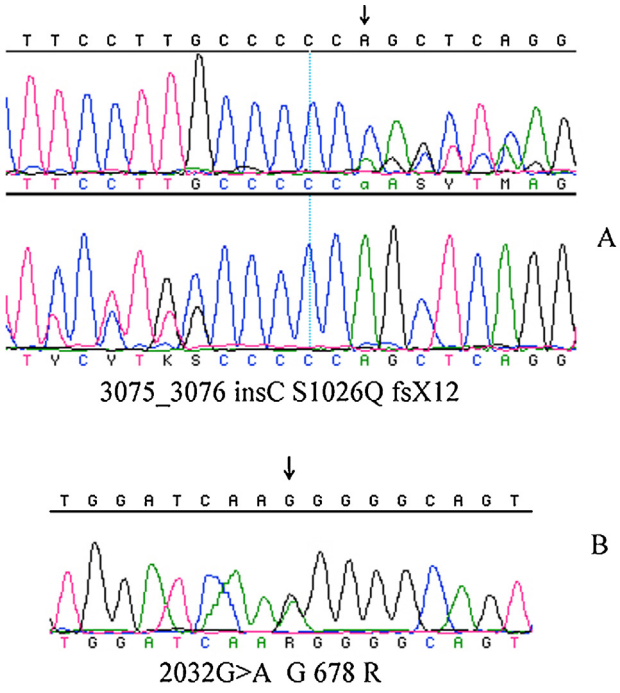

Fig. 1. Mutation type: (A) 21 year-old female showing sense primer sequence (upper) and antisense primer sequence (lower): c.3075_3076 insC. (B) 21 year-old male showing the sense primer sequence: c.2032 G>A.

4. Discussion

In the present study, we found two novel DSG2 mutations in patients dying of ARVC. One was a missense mutation, the pathogenicity of which is uncertain. The other one was insertion mutation, which causing the DSG2 protein truncated, probably pathogenic. We also found two novel DSG2 mutations in patients dying of SUD. Both were missense mutations, the pathogenicity of which are uncertain.

The structural and functional integrity of cardiomyocytes is supported by cell adhesion junctions in the intercalated disc, which contains three types of intercellular connection: desmosomes, adherens junctions and gap junctions. Desmosomes are intercellular adhesive junctions that anchor intermediate filaments and actin cytoskeleton, at the plasma membrane of adjoining cells, providing mechanical attachment between the cells, thereby crucial to tissues that experience mechanical stress, such as epidermis and myocardium. Desmosomes consist of three families of proteins: the cadherin family consisting of transmembrane proteins responsible for anchoring the structure to the

Table 1
DSG2 Mutations.

Cases	Age (yrs), gender	Exon	cDNA	Protein	Novel	Polyphen	SIFT	Mutations Taster
ARVC	21 F	Exon 15	3075_3076 insC	S1026Q fsX12	Yes	–*	–	D
ARVC	21 M	Exon 14	2032G>A	G 678R	Yes	PD	B	B
SUDNA	18 M	Exon 15	2686G>A	E 896K	Yes	PD	B	B
SUDNA	45 M	Exon 15	2573C>T	A 858 V	Yes	B	B	B

M = male, F = female.
PD = possibly damaging.
D = probably damaging.
B = benign.
SIFT = Sorting Intolerant from Tolerant.
–* Indels and nonsense mutations can't be analyzed by PolyPhen and SIFT.