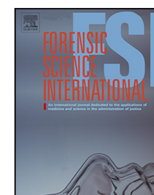
Forensic Science International 270 (2017) 165–172

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint



An insertion/deletion polymorphism within 3′UTR of *RYR2* modulates sudden unexplained death risk in Chinese populations

Shouyu Wang[a,1], Zhixiang Zhang[a,1], Ya Yang[a], Chaoqun Wang[a], Ruiyang Tao[a], Shuxiang Hu[a], Zhixia Yin[a], Qing Zhang[a], Lijuan Li[a], Yan He[b], Shaohua Zhu[a], Chengtao Li[c], Suhua Zhang[c], Jianhua Zhang[c], Lihui Sheng[d], Fangyu Wu[e], Bin Luo[e,*], Yuzhen Gao[a,*]

[a] *Department of Forensic Medicine, Medical College of Soochow University, Suzhou, China*
[b] *Department of Epidemiology, Medical College of Soochow University, Suzhou, China*
[c] *Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Sciences, Ministry of Justice, Shanghai, China*
[d] *Public Security Bureau of Shenzhen, Shenzhen, China*
[e] *Department of Forensic Pathology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China*

ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form 26 October 2016
Accepted 3 December 2016
Available online 9 December 2016

Keywords:
Sudden unexplained death
Ryanodine receptor type-2
rs10692285
Indel polymorphism
Genetic susceptibility

ABSTRACT

Sudden unexplained death (SUD) constitutes a part of the overall sudden death that can not be underestimated. Over the last years, genetic testing on SUD has revealed that inherited channelopathies might play important roles in the pathophysiology of this disease. Ryanodine receptor type-2 (RYR2) is a kind of ion channel extensively distributed in the sarcoplasmic reticulum (SR) of myocardium. Studies on RYR2 have suggested that either dysfunction or abnormal expression of it could lead to arrhythmia, which may cause cardiac arrest. In this study, we conducted a case-control study to evaluate the association of a 4-base pair (4-bp) Indel polymorphism (rs10692285) in the 3′UTR of *RYR2* with the risk of SUD and sudden cardiac death induced by coronary heart disease (SCD-AS) in a Chinese population. Logistic regression analysis showed that the insertion allele of rs10692285 had significantly increased the risk of SUD [OR = 2.03; 95% confidence interval (CI) = 1.08–3.77; P = 0.0161; statistical power = 0.743]. No relevance was observed between rs10692285 and SCD-AS. Further genotype–phenotype association analysis suggested that the expression level of RYR2 in human myocardium tissues with the insertion allele was higher than that with the deletion allele at both mRNA and protein levels. Dual-Luciferase activity assay system was used to detect the effect of rs10692285 on the transcription activity of *RYR2*. As expected, the result indicated that the transcription activity of *RYR2* with the ins/ins genotype was higher than that with the del/del genotype. Finally, *in-silico* prediction revealed that different alleles of rs10692285 could alter the local structure of RYR2 mRNA and microRNA (miRNA) binding. In summary, our findings provided evidence that rs10692285 might contribute to SUD susceptibility through affecting the expression of RYR2, which suggest that abnormal ion channel activity is very likely to be the underlying mechanism of SUD, but not for SCD-AS. Thus, rs10692285 may become a potential marker for molecular diagnosis and genetic counseling of SUD.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Sudden death has long been a threat to the life security of people of all ages, especially the young. Due to the absence of emergency medical response in most area of the world and racial difference, an estimate of its incidence worldwide is currently not available. So far, sudden cardiac death (SCD) is considered to be the major cause of overall sudden death and accounts for at least 10% of the natural mortality in the general population [1,2]. It may attribute to a broad spectrum of cardiac pathologies. Although the vast majority of these may be attributed to coronary atherosclerosis, namely SCD induced by coronary atherosclerosis (SCD-AS), a wide variety of nonatherosclerotic-related cardiac diseases have been also associated with SCD. However, there is a growing part of sudden death victims whose autopsy findings were all negative, including a normal heart structure, and these cases are usually

* Corresponding authors.
E-mail addresses: yuzhengao@suda.edu.cn (Y. Gao), luobin@mail.sysu.edu.cn (B. Luo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.forsciint.2016.12.005
0379-0738/© 2016 Elsevier Ireland Ltd. All rights reserved.

classified as sudden unexplained death (SUD). Due to the difficulties in validating SUD with routine examinations, it is always being an important research field in both clinical medicine and forensic medicine [3–5].

The pathophysiology of SUD is complex and is considered to occur under the interaction between activating events and underlying substrates. With the development of molecular and genetic technology, an increasing number of studies have suggested that abnormal cardiac electrophysiology may be the internal cause of SUD [6–8]. Inherited channelopathies such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome or short QT syndrome (SQTS) had been testified to participate in this fatal disease and the association of genetic variants with SUD occurrence has been widely studied. The mutations in protein-encoding genes vital to cardiac electric activities could be pathogenic [9–11]. Furthermore, Genetic association studies have revealed that common genetic variations in untranslated regions may also contribute to the SUD susceptibility [12–15].

The *RYR2* gene encodes ryanodine receptor type-2 (RYR2), a calcium induced calcium release channel widely found in cardiac cytoplasm, sarcoplasmic reticulum (SR) mainly. These homotetrameric complexes are activated by Ca^{2+} that instantaneously enters the cell through plasma membrane bound L-type calcium channels during depolarization period of the action potential and thus triggers the release of Ca^{2+} stored in the SR through RYR2 that in turn initiates activation of the muscular contraction [16–19]. Previous studies on this gene have revealed that missense mutation in the coding region of *RYR2* is highly relevant to CPVT and sudden cardiac death [20–22]. However, little is known about the effect of polymorphism in the 3′ untranslated region (3′UTR) of *RYR2*. The 3′UTR is considered to be an area with abundant microRNA (miRNA) target regions and could participate in the regulation of gene expression [23,24]. Hence, we chose an Indel polymorphism (rs10692285) within this region from those allele frequencies annotated polymorphisms to analyze its association with SUD as well as SCD-AS susceptibility in a Chinese population, and investigated the possible underlying molecular mechanisms.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Ethical Committee of Soochow University. Written informed consent was obtained from relatives of each participant before the investigation.

2.2. Recruit criteria

In our case-control study, blood samples of SUD and SCD-AS were recruited from Medicolegal Expertise Center of Sun Yat-sen University and Soochow University during 2012–2016. Exhaustive toxicological examinations were performed in all cases to exclude the possibility of poisoning death. Victims were composed of two independent sets, SUD set and SCD-AS set. The 27 SUD cases which had been witnessed living as normal within 24 h before death, suffered a sudden and unexplained death during daily activities or sleep without fatal diseases or violent injuries, and no obvious lethal changes were observed through pathological and histological examinations in comprehensive postmortem autopsy according to the Basso's guidelines for autopsy of forensic investigation [25]. The 51 SCD-AS subjects were presumed to suffer sudden death caused by coronary heart disease since no lethal pathological features but varying degrees of coronary atherosclerosis were detected in these deceased. A total of 298 and 442 healthy controls without any cardiovascular disease history or sudden death family history were frequency matched for age (±5 years) and sex to each set of SUD or SCD-AS cases, respectively. All the controls were recruited from the community nutritional survey conducted in the same regions during the same period as the victims. Additional 18 human myocardium tissues were collected from Medicolegal Expertise Center of Soochow University. To exclude the influence of health condition, gender difference or cause of death, the tissue suppliers were all healthy young male victims died instantaneously in traffic accidents. After medicolegal autopsy, fresh tissues were immediately stored at −80 °C until further process.

2.3. DNA extraction and genotyping

Genomic DNA purification kit (Qiagen) was used to extract the genomic DNA from blood samples. DNA fragments containing rs10692285 were amplified using a pair of genotyping primers (Forward primer: 5′-GCATGTTTATTATGCAAGTT-3′, Reverse primer: 5′-ATACAATTAAGCCCACGA-3′) synthesized by Genewiz Company (Suzhou, China). The PCR products were analyzed by 7% non-denaturing polyacrylamide gel electrophoresis and visualized by silver staining [26]. Genotyping was conducted as described previously in a double-blinded way [27]. The quality control was performed by means of direct sequencing of 50 randomly selected DNA samples in order to validate the genotyping method. Approximately 10% of the total DNA samples selected randomly were examined induplicate by two independent technicians to confirm a 100% consistency.

2.4. Real-time PCR analysis

Total RNA was extracted from human myocardium tissue samples using RNA isolation kit (Cat #74106, Qiagen) and served as templates for reverse transcription, which was performed with Revert Aid First Strand cDNA Synthesis Kit (Cat #K1622, Thermo Scientific). SYBR® real-time PCR (reverse transcriptase-PCR) was performed on Roche Light Cycler 480 system to quantify the relative expression level of *RYR2* mRNA in these samples, and *GAPDH* was chosen as the internal control. Primer sequences used for *RYR2* and *GAPDH* are shown as follows: *RYR2*-F: 5′-GGCAGCCCAAGGGTATCTC-3′, *RYR2*-R: 5′-ACACAGCGCCACCTTCATAAT-3′, *GAPDH*-F: 5′-CTCTCTGCTCCTCCTGTTCGAC-3′, *GAPDH*-R: 5′-TGAGCGATGTGGCTCGGCT-3′. The amplification system was in accordance with those described previously [28]. The $2^{-\Delta\Delta CT}$ algorithm was applied to calculate the expression levels.

2.5. Western blot

Approximately 60 μg of the total protein isolated from human myocardium tissue samples was separated on a 6% polyacrylamide gel, proteins were transferred to a PVDF membrane (GE Healthcare) and probed with primary antibodies Anti-RYR2 (Cat#HPA020028, 1:1500, Atlas Antibodies), Anti-GAPDH (Cat # sc-48167, 1:1000, SantaCruz Biotechnology) and secondary antibody (1:1000, Santa Cruz Biotechnology). After exposure and development, visualized bands were obtained using Enhanced chemiluminescence system (Cell Signaling Technologies) and ImageJ was used to estimate the intensity of those bands which represents the target protein quantity.

2.6. Immunological histological chemistry (IHC)

IHC staining of RYR2 was performed on frozen human myocardium tissue sections as described above using a polyclonal anti-RYR2 antibody (Cat#HPA020028, 1:200, Atlas Antibodies) and a VECTASTAIN Elite ABC Kit (Cat #PK6101, Vector Laboratories) according to the instructions. The sections were treated with Triton

Download English Version:

https://daneshyari.com/en/article/6462541

Download Persian Version:

https://daneshyari.com/article/6462541

Daneshyari.com