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Association between an indel polymorphism in the 3′UTR of *COL1A2* and the risk of sudden cardiac death in Chinese populations

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ABSTRACT

Sudden cardiac death (SCD) describes the unexpected natural death from a cardiac cause within a short time period. Compelling evidence suggests the involvement of host genetic factors in SCD etiology. Identification of genetic variations predisposed to SCD enables genetic testing that may contribute to SCD diagnosis and risk stratification. Previous studies have suggested that dysregulation of pro-alpha2 chain of type I collagen, encoded by collagen type I alpha 2 chain (*COL1A2*) gene, was involved in cardiac disorders such as myocardial infarction, hypertrophic cardiomyopathy and atherosclerosis. By using a candidate-gene-based approach, we evaluated the association of a 7-base pair (7-bp) indel polymorphism (rs3917) in the 3′UTR of *COL1A2* with the risk of SCD in a Chinese population (79 SCD cases and 328 controls). Logistic regression analysis showed that the deletion allele of rs3917 significantly increased the risk of SCD [odds ratio (OR) = 1.82; 95% confidence interval (CI) = 1.08–3.06; $P = 0.0159$]. Further genotype-phenotype association analysis revealed that the deletion allele was markedly correlated with lower expression of *COL1A2* in human myocardium tissues. The luciferase activity analysis in an *in vitro* reporter gene system suggested that rs3917 could regulate *COL1A2* expression through interrupting the binding of miR-296-3p with COL1A2 in an allele-dependent manner, which in turn confer SCD risk. Our data provided initial evidence that rs3917 was highly relevant to SCD susceptibility, and this indel may become a potential marker for molecular diagnosis and genetic counseling of SCD. The replication of our studies and further functional studies are needed to validate our findings.

1. Introduction

Sudden cardiac death (SCD) describes the unexpected natural death from a cardiac cause within a short time period, generally < 1 h from the onset of symptoms, in a person without any prior condition that would appear fatal [1]. SCD is the most common manifestation of coronary heart disease and is responsible for nearly 50% of its victims among people previously not diagnosed with heart disease in developed countries [2]. According to epidemiological analysis, the annual incidence of SCD in China is 41.8/100,000 and it accounted for 43% of all sudden death cases [3,4]. The mechanism of SCD is electrical in nature, but it can also stem from a variety of substrates, with coronary artery disease (CAD) being the most common cause. Besides, primary myocardial diseases such as channelopathies account for most of the residual SCD cases [5]. Although much effort has been put in to study of SCD in last decade, the molecular mechanisms of SCD remain poorly understood and the overall incidence of SCD remains high. With the advancement in scientific knowledge, compelling evidence suggests the involvement of host genetic factors in SCD etiology [6–9]. Therefore, the identification of genetic variations predisposed to SCD is important, since this enables genetic testing that may contribute to SCD diagnosis and risk stratification. Furthermore, the identification of novel genetic risk factors also provides molecular leads which extend the understanding of pathways underlying SCD and development of new prevention and intervention strategies.

Collagen type I alpha 2 chain (*COL1A2*) gene encodes the pro-

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alpha2 chain of type I collagen whose triple helix consists two α1 chains and one α2 chain. As a key component of the extracellular matrix (ECM), type I collagen can be secreted from cardiac fibroblasts and myofibroblasts and represents over 40% of the fibrillar collagen present in both healthy and diseased hearts [10]. Previous studies have identified its roles in common heart disorders such as myocardial infarction [11] and hypertrophic cardiomyopathy [12]. Type I collagen also play important roles during cardiac remodeling if which fails to be inactivated, resulting in fibrosis and cardiac dysfunction [13]. In addition, type I collagen is found in the plaque, the levels of which affect the vulnerability of the plaque and correlate with the mortality and morbidity of atherosclerosis [14,15]. The rupture of atherosclerotic plaque remains a leading cause of acute cardiovascular death [16]. Therefore, dysregulation of COL1A2 may have implications in SCD. Our previous studies have identified a 7-bp indel polymorphism (rs3917) within 3′UTR of COL1A2 and investigated its associations with hepatocellular cancer susceptibility [17]. In the current case control study, by using a candidate-gene-based approach, we evaluated the association between rs3917 and SCD susceptibility in Chinese populations. Additional experimental and *in-silico* studies were used to assess the possible functional significance of this polymorphism.

2. Materials and methods

2.1. Study populations

Our study included an independent case-control set containing 79 SCD cases and 328 controls which were genetically unrelated ethnic Han Chinese. The blood samples of SCD were recruited from Medicolegal Expertise Center of Soochow University, Sun Yat-sen University, Xiangya Medical University and Institute of Forensic Science, Ministry of Justice during 2012–2016. Elaborate toxicological examinations were implemented in all cases to exclude the possibility of toxic death. The 79 SCD subjects were presumed to suffer sudden death caused by coronary heart disease, since no obvious lethal changes were observed except for varying degrees of coronary atherosclerosis. A total of 328 healthy controls without any cardiovascular disease history or sudden death family history were frequency matched for age (± 5 years) and sex to SCD cases. All the controls were recruited from the community nutritional survey conducted in the same regions during the same period as the victims. The additional 23 human myocardium tissues were collected from Medicolegal Expertise Center of Soochow University, after medicolegal autopsy, and immediately stored at −80 °C till use. To exclude the influence of health condition, sex difference or cause of death, the tissue suppliers were all healthy young male victims died unexpectedly in traffic accidents. 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. DNA extraction and genotyping

Genomic DNA purification kit (Qiagen) was used to extract the genomic DNA from blood samples. DNA fragments containing rs3917 were amplified using a pair of genotyping primers (Forward primer: 5′-CTGTGGAACCATGGAAGAAG-3′, Reverse primer: 5′-GTATTGAG TTGTATCGTGTGG-3′). The PCR products were analyzed by 7% non-denaturing polyacrylamide gel electrophoresis and visualized by silver staining [18]. Genotyping was conducted as described previously in a double-blinded way [19]. The quality control was done as follow: 50 randomly selected DNA samples were sequenced following genotyping 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.

2.3. Real-time PCR analysis

Total RNA was extracted from human myocardium tissue samples according to the manufacturer's protocol (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 was performed on Roche Light Cycler 480 system to quantify the relative expression level of *COL1A2* mRNA in these samples, and *GAPDH* was chosen as the internal control. Primer sequences used for *COL1A2* and *GAPDH* are shown as follows: COL1A2-F: 5′-GGCCCTCAAGGTTTCCAAGG-3′, COL1A2-R: 5′-CACCCTG TGGTCCAACAACTC-3′, GAPDH-F: 5′-CTCTCTGCTCCTCCTGTTCGAC-3′, GAPDH-R: 5′-TGAGCGATGTGGCTCGGCT-3′. The amplification system was in accordance with those described previously [20]. The $2^{-\Delta\Delta CT}$ algorithm was applied to calculate the expression levels.

2.4. In-silico analysis

The hybridization of putative miRNA and the *COL1A2* 3′UTR harboring either the 7-bp insertion or deletion allele were predicted by miRanda with default parameters [21].

2.5. Cell culture, construction of vector and luciferase reporter assay

In vitro experiments were performed as previously described [22]. Briefly, 293 T cell lines were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C. The cell lines were characterized by Genetic Testing Biotechnology Corporation (Suzhou, China) using short tandem repeat (STR) markers.

A total of 307-bp fragments containing the deletion allele of rs3917 were directly synthesized by Genewiz Company (Suzhou, China) and subcloned into XbaI and FseI sites of pGL3-control vector, generating the wild type vector (pGL3-COL1A2-WT) and the mutant type vector containing the insertion allele (pGL3-COL1A2-MT). The resultant constructs were verified by direct sequencing.

Cells were seeded in 24-well plates at a density of 1×10^5 cells per well. Twenty-four hours after plated, cells were transfected by vectors with the cooperation of Lipofectamin 2000 according to manufacturer's protocol. Approximately 400 ng reconstructed vector pGL3-COL1A2-WT or pGL3-COL1A2-MT was co-transfected with 30 ng pRL-CMV vector (Promega) in each well, and the empty pGL3-control vector co-transfected group was performed as negative control. Additional 40 nmol miR-296-3p mimic or negative mimic controls were added in each well, respectively. Twenty-four hours after transfection, cells were harvested immediately with addition of 100 mL passive lysis buffer. Firefly luciferase activity in cell lysate was measured in FilterMaxF5 (Molecular Devices) using the Dual Luciferase assay system and normalized with the Renilla luciferase activity. Each group was duplicated in 6 wells and every experiment was repeated at least 3 times.

2.6. Statistical analysis

The genotype distribution was analyzed by Hardy-Weinberg equilibrium using chi-square test. Logistic regression was used to assess the associations between the rs3917 and SCD risk, adjusted by sex and age. The relative expression levels of COL1A2 in tissue samples with different genotypes were compared using nonparametric *Mann-Whitney* test. Student's *t* test was used to examine the differences in luciferase reporter gene expression. These statistical analyses were implemented by Statistic Analysis System software (version 8.0, SAS Institute), $P < 0.05$ was used as the criterion of statistical significance. All statistical tests were two sided. The statistical power of the current sample size was calculated using the G*Power 3.1 software [23].