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Cardiac channelopathy testing in 274 ethnically diverse sudden unexplained deaths

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ABSTRACT

Sudden unexplained deaths (SUD) in apparently healthy individuals, for which the causes of deaths remained undetermined after comprehensive forensic investigations and autopsy, present vexing challenges to medical examiners and coroners. Cardiac channelopathies, a group of inheritable diseases that primarily affect heart rhythm by altering the cardiac conduction system, have been known as one of the likely causes of SUD. Adhering to the recommendations of including molecular diagnostics of cardiac channelopathies in SUD investigation, the Molecular Genetics Laboratory of the New York City (NYC) Office of Chief Medical Examiner (OCME) has been routinely testing for six major channelopathy genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and RyR2) since 2008. Presented here are the results of cardiac channelopathy testing in 274 well-characterized autopsy negative SUD cases, all with thorough medicolegal death investigation including complete autopsy by NYC OCME between 2008 and 2012. The cohort consisted of 141 infants (92.9% younger than six-month old) and 133 non-infants (78.2% were between 19 and 58 years old). Among the ethnically diverse cohort, African American infants had the highest risks of SUD, and African American non-infants died at significantly younger age (23.7 years old, mean age-at-death) than those of other ethnicities (30.3 years old, mean age-at-death). A total of 22 previously classified cardiac channelopathy-associated variants and 24 novel putative channelopathy-associated variants were detected among the infants (13.5%) and non-infants (19.5%). Most channelopathy-associated variants involved the SCN5A gene (68.4% in infants, 50% in non-infants). We believe this is the first study assessing the role of cardiac channelopathy genes in a large and demographically diverse SUD population drawn from a single urban medical examiner's office in the United States. Our study supports that molecular testing for cardiac channelopathy is a valuable tool in SUD investigations and provides helpful information to medical examiners/coroners seeking cause of death in SUD as well as potentially life-saving information to surviving family members.

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1. Introduction

Sudden unexplained death (SUD) in apparently healthy individuals presents vexing challenges for medical examiners and still remains an important public health priority. SUD is defined by a cause of death which remains unknown after complete autopsies, comprehensive laboratory testing, review of all available history, and performance of a comprehensive scene investigation. According to the Centers for Disease Control and Prevention, more than 4500 infants die suddenly each year in the United States with no immediate or obvious cause of death; half of those deaths remain unexplained after a forensic investigation. Furthermore, the prevalence of sudden unexplained deaths beyond infancy (1–22 years old) is also estimated to be greater than 2000 annually in the United States [1]. Despite a decline in SUD rates over the past decade, the rates are still disproportionately high

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amongst certain population groups, especially in African Americans and American Indian/Alaska Natives [2].

It is estimated that 10–35% [3–8] of SUD may be explained by cardiac channelopathies, which affect heart rhythm and cardiac electrical conduction physiology. These disorders comprise of a group of inheritable cardiac arrhythmia syndromes, such as long QT syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), Brugada syndrome, and short QT syndrome. Despite extensive attempts at developing nationalized standards in SUD investigation including the establishment of basic guidelines [9,10], currently, wide variation exists across the United States when investigating and certifying SUD [11]. Unfortunately, the use of molecular diagnostics is not commonly made as part of the medical investigation of SUD cases. The situation in European countries is also similar, with the application of genetic testing in routine forensic investigations being very limited [12]. Nevertheless, the New York City (NYC) Office of Chief Medical Examiner (OCME) has routinely integrated molecular testing of six major cardiac channelopathy genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and RyR2) in SUD investigations since 2008. This testing panel is significant for over 80% of the long QT syndromes, 25% of Brugada syndrome, and 50% of catecholaminergic polymorphic ventricular tachycardia (CPVT) [13].

The main goal of this study is to evaluate the significance of cardiac channelopathies in SUD within a large and ethnically diverse population from a single urban medical examiner's office in the United States.

2. Materials and methods

2.1. Study cohort

Biological samples from a total of 340 cases of sudden unexpected natural death were submitted to the Molecular Genetics Laboratory in the New York City OCME for cardiac channelopathy testing from 2008 to 2012. Of those cases, 66 cases were excluded from this study based on the following criteria: (1) only an external examination was performed (often due to religious objection to autopsy), and (2) the causes of deaths were explained by autopsy or forensic laboratory findings. Based on this, 274 autopsy negative cases were included in this study. All 274 cases went through a comprehensive forensic investigation, including scene investigation, police investigation, full-autopsy with microscopic examinations of heart and central nervous system, ancillary studies (including toxicology, metabolic screening in all infants, microbiology), andareview of the clinical history when available. Molecular testing for six channelopathy genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and RyR2) was performed by the Molecular Genetics Laboratory (accredited by College of American Pathologists).

2.2. Molecular testing

Molecular analyses were performed for six major cardiac channelopathy genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 and RyR2) in which disease-causing sequence variants have been previously reported in SUD. Specifically, the panel contained the entire coding regions of the KCNQ1 (NM_000218.2), KCNH2 (NM_000238.2), SCN5A (NM_198056.2), KCNE1 (NM_000219.3), KCNE2 (NM_172201.1) genes, and selected exons of the RyR2 (NM_001035.2) gene (exon 8, 14, 15, 44–47, 49, 88–93, 95–97, 100–105) in 274 cases.

Genomic DNA was extracted and purified from postmortem tissues or dried blood cards using standard extraction techniques on the M48 BioRobot (Qiagen, CA). Next-generation sequencing (NGS) method was used for testing exons 2–11 in SCN5A and Sanger sequencing was used to test the other genes and to perform confirmation of identified variants. For next generation sequencing method, the probes were designed by illumina design studio, and the library preparation was performed using the TruSeq Custom Amplicon Library Preparation Kit v1.5 (Illumina, San Diego, CA, USA) according to manufacturer's instructions. The genomic DNA input for each sample was from 11 ng to 220 ng. The Illumina ACD-1 and in-house positive control samples with known variants in SCN5A exons 2–11 were used as positive controls, and Milli-Q water was used as negative control for each 96-well reaction plate. 5% Phix was spiked-in to the library to monitor the quality of the cluster generation and sequencing reaction. Sequencing was performed on the MiSeq sequencer v2 with the MiSeq Reagent Nano Kit v2 (300 cycles) (Illumina, San Diego, CA, USA) for each plate of up to 96 samples according to the manufacturer's instructions (MiSeq System user guide). Avadis-NGS commercial software (v1.5.1) was used for NGS data analysis. More than 99% target regions with coverage higher than 300 reads, which were aligned to human genome reference (UCSC hg19) using BWA aligner. Non-synonymous variants with possible damaging effects were validated by conventional Sanger sequencing method.

For Sanger sequencing, the exons and intron–exon boundaries were amplified and directly sequenced using big-dye terminator chemistry and automated capillary electrophoresis system 3130xl from Applied Biosystems Life Technologies (Carlsbad, CA). Sequencing data were analyzed by Sequencher 4.9 (Gene Codes Corporation, MI). The nucleotide sequence variants were denoted using Human Genome Variation Society (HGVS)-recommended nomenclature [14].

2.3. Data analysis

The study cohort of 274 cases was divided into two main age groups: infants ($\leq$1 year old group), and non-infants ($>$1 year old group). Demographic characteristics of the cohort are described in Table 1 and Fig. 1. Previously classified cardiac channelopathy associated variants and novel putative cardiac channelopathies variants were re-evaluated utilizing the recently expanded Exome Sequencing Project (ESP) database [15] and the 1000 genomes database [16], as well as the functional prediction programs, Polyphen 2 [17] and SIFT [18] (Table 2). The roles of the putative cardiac channelopathy associated variants were evaluated by age groups (Tables 3 and 4). The distributions of the common variants in this study cohort were compared to those reported in the ESP database [15] (Table 6). Pearson's Chi-square test or *t*-test was performed to assess the statistical significance of the categorical variables or continuous data. The Kaplan–Meier cumulative survival curve was used to evaluate the age-at-death by ethnicity. The Log Rank test and two-tailed *t*-test were used to evaluate the age-at-death differences among all ethnicities, and between two ethnicities, respectively. Statistical significance is indicated by $p < 0.05$. SPSS and GraphPad Prism were used for statistical analysis.

3. Results

3.1. Unique demographic characteristics of the study cohort

The demographic characteristics of the 274 sudden unexplained deaths (SUD) investigated by the NYC OCME are summarized in Table 1. These 274 cases were divided into two age groups: infants ($\leq$1 year old, 141 cases) and non-infants ($>$1 year old, 133 cases). In infants, 92.9% were younger than 6 months. In non-infants, 78.2% were older than 18 years old (the oldest decedent was 58 years old). African American is the leading ethnicity observed in both infants (60.3%) and non-infants (35.3%).