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Post-mortem genetic analysis in juvenile cases of sudden cardiac death

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ABSTRACT

Background: The reason behind a sudden death of a young individual remains unknown in up to 50% of postmortem cases. Pathogenic mutations in genes encoding heart proteins are known to cause sudden cardiac death.
Objective: The aim of our study was to ascertain whether genetic alterations could provide an explanation for sudden cardiac death in a juvenile cohort with no-conclusive cause of death after comprehensive autopsy.
Methods: Twenty-nine cases <15 years showing no-conclusive cause of death after a complete autopsy were studied. Genetic analysis of 7 main genes associated with sudden cardiac death was performed using Sanger technology in low quality DNA cases, while in good quality cases the analysis of 55 genes associated with sudden cardiac death was performed using Next Generation Sequencing technology.
Results: Thirty-five genetic variants were identified in 12 cases (41.37%). Ten genetic/variants in genes encoding cardiac ion channels were identified in 8 cases (27.58%). We also identified 9 cases (31.03%) carrying 25 genetic variants in genes encoding structural cardiac proteins. Nine cases carried more than one genetic variation, 5 of them combining structural and non-structural genes.
Conclusions: Our study supports the inclusion of molecular autopsy in forensic routine protocols when no conclusive cause of death is identified. Around 40% of sudden cardiac death young cases carry a genetic variant that could provide an explanation for the cause of death. Because relatives could be at risk of sudden cardiac death, our data reinforce their need of clinical assessment and, if indicated, of genetic analysis.

1. Introduction

Sudden death in people younger than 15 years old is a rare event, with an incidence between 1–5/100,000 individuals each year in developed countries [1]. Despite this low prevalence, when a death occurs in this juvenile population, it carries a tremendous impact in both the family and community. Sudden death constitutes one of the most important unsolved challenges in the practice of forensic pathology. Several studies have reported that most part of sudden deaths in the young (<40 years) is of cardiac origin (sudden cardiac death -SCD-), mainly caused by structural heart abnormalities identifiable at autopsy (cardiomyopathies) [2]. However, in 10–35% of these deaths, no structural alterations can be identified. In these cases a channelopathy, a genetic disease of the cardiac ion channels, is suspected [3–5]. Both groups of cardiac alterations are due to inherited genetic defects, thus family members of the deceased individual are at risk of sudden death [6]. This fact carries important implications in diagnosis and counselling of relatives. Though, the application of genetic testing in routine forensic investigation, to benefit diagnosis and possible family prevention, remains still very limited [7].

Currently, numerous genes have been associated with SCD but most part in low frequency [8,9]. However, in these last years,

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genetic research has focused on the identification of pathogenic mutations in seven main genes (*SCN5A, KCNQ1, KCNH2, KCNE1, KCNE2, KCNE3*, and *RyR2*) associated with channelopathies (like Brugada Syndrome-BrS-, Long QT Syndrome -LQTS-, Short QT Syndrome -SQTS-, and Catecholaminergic Polymorphic Ventricular Tachycardia -CPVT-) and 7 main genes (*MYBPC3, MYH7, PKP2, DSC2, DSP, DSC2*, and *LMNA*) associated with cardiomyopathies (like Hypertrophic Cardiomyopathy -HCM-, Arrhythmogenic Right Ventricular Cardiomyopathy -ARVC-, and Dilated Cardiomyopathy -DCM-).

Genetic analysis of these genes can help in the identification of the cause of death, even using mRNA [10], improving the evaluation of relatives at potential risk. Traditional Sanger sequencing is expensive to undertake this extensive analysis. However, new genetic technologies (Next generation Sequencing -NGS-) have emerged as a cost-effective technology for broad genetic studies [11–13]. The ability to perform analysis of large amount of genes at once has been brought to the clinical arena of several medical specialities, including cardiology. It is no secret though, that the large amount of data generated is causing difficulties in clinical interpretation, especially when dealing with genetic variants of unknown significance (GVUS) or genetic variants in less common genes. In our study we analyzed a cohort of post-mortem cases, aged less than 15 years old, in order to investigate the role of genetics in death causality.

2. Methods

2.1. Forensics

A complete autopsy examination was performed according to current international regulations [14]. Our inclusion criteria was: (a) age <15 years, (b) non-conclusive cause of death after complete autopsy, (c) no signs of congenital heart alterations, cardiac infarct or other macroscopic anomalies, (d) blood obtained <48 h after death. The study was approved by the ethics committee of our Hospital, and follows the Helsinki II declaration.

2.2. DNA sample

Genomic DNA was extracted with Chemagic MSM I from post-mortem whole blood (Chemagic human blood). DNA cases were checked in order to assure quality (absorbance 260/280:260/230 should be a minimum 1.8:2.2, respectively), and quantify before processing to get the 3 μg needed for the NGS strategy. Spectrophotometric measurements are performed to assess quality ratios of absorbance; DNA concentration is determined by fluorometry (Qubit, Life Technologies). DNA integrity was assessed on a 0.8% agarose gel.

DNA quality/integrity divided our cohort of 29 cases in two groups. The first group included 18 cases with low DNA quality/integrity and analyzed using Sanger sequencing (*SCN5A, KCNQ1, KCNH2, KCNE1, KCNE2, KCNE3* and *RyR2*). The second group included 11 cases analyzed using NGS technology (55 genes associated with SCD). Confirmation of variants identified in NGS analysis was performed using Sanger sequencing. As an internal control, two cases included in the second group were processed by both methods.

2.3. Sanger sequencing

The genetic study included direct sequencing of *SCN5A* (NM_198056), *KCNQ1* (NM_000218), *KCNH2* (NM_000238), *KCNE1* (NM_000219), *KCNE2* (NM_172201), *KCNE3* (NM_005472), and *RyR2* (NM_001035). The exons and exon–intron boundaries of each gene were amplified (Verities PCR, Applied Biosystems, Austin, TX, USA), the PCR products were purified (Exosap-IT, Affymetrix, Inc. USB® Products, Cleveland, OH, USA) and they were directly sequenced in both directions (Big Dye Terminator v3.1 cycle sequencing kit and 3130XL Genetic Analyzer, both from Applied Biosystems) with posterior SeqScape Software v2.5 (Life Technologies) analysis comparing obtained results with the reference sequence from hg19. The identified variations were compared with DNA sequences from 300 healthy Spanish individuals (individuals not related to any patient and of the same ethnicity; 600 alleles), as control cases, and contrasted with Human Gene Mutation Database -HGMD- (http://www.hgmd.cf.ac.uk/ac/index.php), HapMap (http://hapmap.ncbi.nlm.nih.gov/), 1000 genomes project (http://www.1000genomes.org/), and Exome Variant Server -EVS- (http://evs.gs.washington.edu/EVS/). Sequence variants were described following the HGVS rules (http://www.hgvs.org/), and checked in Mutalyzer (https://mutalyzer.nl/).

2.4. NGS sample preparation

The DNA was fragmented by Bioruptor (Diagenode). Library preparation was performed according to the manufacturer's instructions (SureSelect XT Custom 0.5–2.9 Mb library, Agilent Technologies, Inc.). After capture, the indexed library was sequenced in a six-sample pool cartridge. Sequencing paired-end process was developed on MiSeq System (Illumina) using 2× 150 bp reads length.

2.5. Custom resequencing panel

We selected the most prevalent 55 genes involved in SCD-related pathologies, accordingly to available scientific literature [8,9]. The genomic coordinates corresponding to these 55 genes (Table 1) were designed using the tool eArray (Agilent Technologies, Inc.). All the isoforms described at the UCSC browser were included in the design. The biotinylated cRNA probe solution was manufactured by Agilent Technologies and provided as capture

Table 1
List of the 55 SCD-related genes included in our panel and its association with the disease. Some genes are associated with more than one disease.

Disease	Genes
Brugada Syndrome	*CACNA1C, CACNB2, GPD1L, HCN4, SCN5A*
Long QT Syndrome	*ANK2, CACNA1C, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, SCN4B, SCN5A*
Short QT Syndrome	*CACNA1C, CACNB2, KCNH2, KCNJ2, KCNQ1*
Catecholaminergic Polymorphic Ventricular Tachycardia	*CASQ2, KCNJ2, RYR2*
Hypertrophic Cardiomyopathy	*ACTC1, ACTN2, CAV3, CSRP3, GLA, JPH2, LAMP2, LDB3, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, PDLIM3, PLN, PRKAG2, RYR2, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL*
Dilated Cardiomyopathy	*ACTC1, ACTN2, CAV3, CRYAB, CSRP3, DES, DMD, DSC2, DSG2, DSP, EMD, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, PKP2, PLN, SCN5A, SGCD, TAZ, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL*
Arrhythmogenic Right Ventricular Cardiomyopathy	*DES, DSC2, DSG2, DSP, JUP, LMNA, PKP2, PLN, TGFB3, TTN*