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The role of clinical, genetic and segregation evaluation in sudden infant death

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ABSTRACT

Sudden infant death syndrome (SIDS) is the leading cause of death in the first year of life. Several arrhythmogenic genes have been associated with cardiac pathologies leading to infant sudden cardiac death (SCD). Our aim was to take advantage of next generation sequencing (NGS) technology to perform a thorough genetic analysis of a SIDS case.

A SIDS case was referred to our institution after negative autopsy. We performed a genetic analysis of 104 SCD-related genes using a custom panel. Confirmed variants in index case were also analyzed in relatives. Clinical evaluation of first-degree family members was performed.

Relatives did not show pathology. NGS identified seven variants. Two previously described as pathogenic. Four previously catalogued without clinical significance. The seventh variation was novel. Familial segregation showed that the index case's mother carried all same genetic variations except one, which was inherited from the father. The sister of the index case carried three variants.

We believe that molecular autopsy should be included in current forensic protocols after negative autopsy. In addition to NGS technologies, familial genetic testing should be also performed to clarify potential pathogenic role of new variants and to identify genetic carriers at risk of SCD.

1. Introduction

Sudden infant death syndrome (SIDS) is defined as the death of an apparently healthy infant of less than one year of age. The death usually occurs during sleep and remains unexplained after an exhaustive investigation including complete autopsy and medical history [1]. Despite SIDS rates differ significantly across countries, ethnic groups and gender [2], SIDS is the main cause of death in Europe and North-America in infants less than one year of age [3]. A large number of pathophysiological mechanisms have been suggested but the etiology of SIDS still remains to be clarified. SIDS is considered a multifactorial disorder, with several intrinsic and extrinsic risk factors resulting in or predisposing to the death of the infant. Among them, genetic defects associated with inheritable arrhythmias play a role in this entity [4]. To date, 10–15% of the SIDS cases are thought to be caused by cardiac channelopathies [5].

The 'molecular autopsy' enables genetic analysis to identify the defect that might be associated with a certain disease [6,7]. Until now, few molecular autopsy series have been reported [8]. In addition, these studies have included the analyses of only the major long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT) genes using a candidate gene approach [4,9]. Financial limitations have impaired the use of this technology beyond the research area. The advent of massive parallel DNA sequencing platforms, named next-generation sequencing (NGS) technology, has revolutionized the field of medical genomics, allowing fast and cost-effective generation of genetic data [10]. The massive genetic screening has yet to fully enter the clinical field, hampered by the excess of generated genetic data, and specially the clinical phenotype interpretation.

The purpose of this study was to identify the genetic defect that could explain the cause of death in a SIDS case. Due to the amount

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of genes associated with lethal arrhythmogenic syndromes, we used a NGS custom panel. To our knowledge, no genetic report has been performed in SIDS cases using custom panel technology.

2. Methods

2.1. Forensics and clinics

A complete autopsy examination was performed according to current international regulations for unexpected death [11–13]. All relatives included in our study were clinically evaluated at Hospital Josep Trueta of Girona (Girona, Spain), and Hospital Sant Joan de Déu (Barcelona, Spain). Complete clinical evaluation, including electrocardiogram (ECG) and echocardiogram (ECHO), was performed in index case's parents and sister. The study was approved by the ethics committee of the Hospital Josep Trueta (Girona, Spain), followed the Helsinki II declaration and informed consent was obtained from all participants. All patients were Caucasian and native of Spain.

2.2. DNA sample

Genomic DNA was extracted with Chemagic MSM I from whole blood (Chemagic human blood). DNA samples were checked in order to assure quality and quantify before processing to get the 3 μg needed for the NGS strategy. DNA integrity was assessed on a 0.8% agarose gel. Spectrophotometric measurements are also performed to assess quality ratios of absorbance; dsDNA concentration is determined by fluorometry (PicoGreen assay). DNA sample was fragmented by Adaptive Focused Acoustics (Covaris). Library preparation was performed according to the manufacturer's instructions (SureSelect XT Custom 0.5–2.9 Mb library, Agilent Technologies, Inc.). Indexed libraries enter finally in the sequencing path; pooled captures (up to 13 samples per lane) were sequenced on Illumina HiSeq2000 instrument using 2 bp × 75 bp reads length.

2.3. Custom resequencing panel

We selected the most prevalent 104 genes involved in SCD-related pathologies, accordingly to available scientific literature focus on SCD, so far. The genomic coordinates corresponding to these 104 genes (Table 1) were designed by Ferrer inCode using the tool eArray (Agilent Technologies, Inc.). All the isoforms described at the University of California, Santa Cruz (UCSC) browser were included at the design. The biotinylated cRNA probe solution was manufactured for Ferrer inCode by Agilent Technologies and provided as capture probes. The final size was 680 Kbp of encoding regions and UTR boundaries. The coordinates of the sequence data is based on National Center for Biotechnology Information (NCBI) build 37 (UCSC hg19).

2.4. Bioinformatics

The bioinformatic approach includes a first step trimming of the FAST-Q files with a Ferrer inCode developed method. The trimmed reads are then mapped with GEM II and output is joined and sorted and uniquely and properly mapping read pairs are selected. Finally, variant call over the cleaned BAM file is performed with SAMtools v.1.18, GATK v2.4 together with a Ferrer inCode developed method to generate the first raw VCF files. Variants are annotated with dbSNP IDs, Exome Variant Server and the 1000 genomes browser, in-home database IDs and Ensembl information, if available.

Tertiary analysis is then developed. For each genetic variation identified, allelic frequency was consulted in Exome Variant Server – EVS – (http://evs.gs.washington.edu/EVS/) and 1000 genomes database (http://www.1000genomes.org/). In addition, Human Gene Mutation Database – HGMD – (http://www.hgmd.cf.ac.uk/ac/index.php) was also consulted to identify pathogenic mutations previously reported. *In silico* pathogenicity of novel genetic variations were consulted in CONDEL software (CONsensus DELeteriousness scores of missense SNVs) (http://bg.upf.edu/condel/), and PROVEAN (PROtein Variation Effect ANalyzer) (http://provean.jcvi.org/index.php). Alignment among species was also performed for these novel variations using Uniprot database (http://www.uniprot.org/).

2.5. Genetic confirmation

Pathogenic known mutations and rare genetic variants were confirmed by Sanger method. First, polymerase chain reaction (PCR) was performed. PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH, USA), and the analysis of the exonic and intron-exon regions was performed by direct sequencing (Genetic Analyzer 3130XL, Applied Biosystems) with posterior SeqScape Software v2.5 (Life Technologies) analysis comparing obtained results with the reference sequence from hg19. Each sample underwent a genetic study of corresponding genes (NCBI – National Center for Biotechnology Information – http://www.ncbi.nlm.nih.gov/) (*TTN* NM_133378, *EN1* NM_001426, *AKAP9* NM_005751, *VCL* NM_014000, *KCNE3* NM_005472, *PKP2* NM_004572). Familial cosegregation of rare genetic variants was also performed using Sanger technology.

3. Results

3.1. Forensic and clinical data

The 11 month-old male was born full term with uneventful antenatal and perinatal history. During his months of life, no anomalous clinical events were reported, including syncopes, infections, metabolic disorders, or epileptic episodes. The death occurred at night, during sleep. Scene investigation did not reveal any relevant detail. A comprehensive autopsy was performed, revealing that all organs were normal in size and structure, with no evidence of trauma, malignancy, malformation, infection or metabolic disease. Toxicological test and histological study did not reveal any anomalous substance or microscopic alteration, respectively. The forensic conclusion was unexpected death of unknown cause after a thorough investigation, in concordance to San Diego classification of SIDS [14]. The parents were non-consanguineous and neither them of their families showed any previous history of any pathology associated to SD. All relatives were clinically assessed (father, mother and sister). The clinical

Table 1
List of the 104 SCD-related/suspicious genes included in our panel.

ABCC9, ACTA2, ACTC1, ACTN2, AKAP9, ANK2, CACNA1C, CACNA1G, CACNA1H, CACNA1I, CACNB2, CASQ2, CAV3, CHRM2, CRYAB, CSRP3, CTF1, DES, DMD, DSC2, DSG2, DSP, ECE1, EMD, EN1, EYA4, FBN1, FHL2, FKTN, GJC1 (GJA7), GLA, GPD1L, HCN1, HCN2, HCN4, ILK, JPH2, JUP, KCNA4, KCNA5, KCND2, KCND3, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ3, KCNK4, KCNQ1, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPN, NEXN, NOS1AP, NPPA, NUP155, PDLIM3, PHOX2A, PHOX2B, PKP2, PLN, PRKAG2, PSEN1, PSEN2, RBM20, RET, RYR2, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SGCA, SGCB, SGCD, SIRT3, SLC25A4, SLC6A4 (5HTT), SLC8A1, SNTA1, TAZ, TCAP, TGFB3, TGFBR1, TGFBR2, TLX3, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TPM1, TTN, VCL