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Study by next generation sequencing of sudden cardiac death (SCD)

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ABSTRACT

Sudden cardiac death (SCD) is one of the most common causes of death; most SCD are related to secondary arrhythmias, to structural heart disease, or to primary electrical abnormalities of the heart.

A significant number of SCD, especially among young people, are due to genetic heart disorders, both with structural and arrhythmogenic abnormalities. However SCD occurs also in patients with negative clinical history, autopsy is not always conclusive for a diagnosis.

Recent technological advances in DNA sequencing, have led to the commercialization of genetic testing now widely available in clinical practice. In particular, next generation sequencing, allows the large-scale and rapid assessment of entire genomes.

Analysis of SCD with a NGS panel of 174 genes was performed in our laboratory in order to identify the genetic causes and thus to direct the clinician to an accurate clinical and genetic screening of relatives.

Two SDC were studied:

Case 1: female, 57, without story of syncope and no previously highlighted cardiac alteration, died post cardiac arrest; negative family history. Autopsy was apparently negative.

Case 2: male, 52, who died during a football game; negative family history, neurological episodes occurred before death was reported by close relative. Autopsy was positive for ventricular hypertrophic.

In both cases we made a genetic diagnosis.

1. Introduction

Sudden death (SD) is defined as "unexpected death, which occurs within an hour of the onset of acute symptomatology, in subjects in full well-being or in subjects whose chronic disease status does not provide for such a sudden outcome". An estimated 70,000 people die suddenly in Italy each year. For the purpose of limiting the concept of sudden death to heart diseases, the word "cardiac" was added but it is important to distinguish between coronary and non-coronary. About 80% of patients who died of sudden death reported coronary artery disease. Cardiomyopathies are the second most important cause of sudden cardiac death (SCD).

SCD can be a consequence of genetic anomalies that include both arrhythmic and structural diseases such as Long QT syndrome (LQTS), Brugada syndrome (BS), Catecholaminergic polymorphic ventricular tachycardia (CPVT), Hypertrophic cardiomyopathy (HCM), Arrhythmogenic cardiomyopathy of right ventricle (ARVC), Dilated cardiomyopathy (DCM).

From large-scale epidemiological studies emerges that genetic factors are at the basis of a greater familial incidence of SCD [1] so, genetic counselling to the relatives of decesead probands and, eventually, their cardiological and genetic screening, becomes fundamental.

NGS methods and platforms have matured during the last 10 years, and the quality of the sequences has reached a level where NGS is used in clinical diagnostics of humans. Forensic genetic laboratories have also explored NGS technologies and especially in the last year, there has been a small explosion in the number of scientific articles and presentations at conferences with forensic aspects of NGS. These contributions have demonstrated that NGS offers new possibilities for forensic genetic case work.

In this study we therefore used the NGS technique in a case of sudden death in which the autopsy data was apparently negative (Case 1) and in a case where the autopsy had revealed the suspicion of CMI (Case 2).

The cases have been sent to Laboratory of Genetic Diagnostics by the Legal Medicine after the autopsy.

Genetic counseling was carried out with both families in order to collect family history and informed consent to performe genetic test in the deceased probands.

Case 1: female, 57, without story of syncope and no previously highlighted cardiac alteration, died post cardiac arrest; negative family history. Autopsy was apparently negative. She had a son of 23 years old.

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Case 2: male, 52, who died during a football game; negative family history, neurological episodes occurred before death was reported by close relative. Autopsy was positive for ventricular hypertrophy. He had a sister of 54 years old with two sons of, respectively, 18 and 20 years old.

2. Material and methods

The genetic analyses was conducted with the prior consent of the relatives of deceased subjects.

Total genomic DNA was extracted for Case 1 from peripheral blood leukocytes and for Case 2 from paraffin embedded cardiac muscular tissue, according to manufacturer's recommendations (EZ1 kit Qiagen,Hilden, Germany).

Sequence-targeted libraries were prepared according to manufacturer's recommendations (Trusight Cardio, Illumina, San Diego CA, USA) and sequenced on Next Seq 500 (Illumina San Diego CA, USA).

Sequenced reads were mapped against the human reference genome hg19 by BWA (version 0.6.2) [2].

The alignment files were subjected to quality score recalibration and local realignment around indels by using Genome Analysis ToolKit (GATK) (version 1.6.11) [3]. Single Nucleotide Variants (SNVs) and Insertion/Deletions (InDels) were identified by the UnifiedGenotyper module of GATK and functionally annotated by ANNOVAR [4].

BEDTools suite (version .217.0) [5] was used to identify target regions with coverage < 20X that were considered as not adequately covered and therefore excluded from the analysis.

From the annotation variant files, we only retained variants in coding exons and flanking exon/intron of genes. Synonymous variants and variants reported in the population databases with frequency above 1%, were excluded. The selection of variants have been made on the basis of the following criteria: 1) consistency with the clinical suspicion and the inheritance pattern expected; 2) the presence of the variant in the database of pathology (dbSNP135 Release [http://www.ncbi.nlm.nih.gov/SNP/], Exome sequencing Project 6500 ESP [http://evs.gs.washington.edu/EVS/], 1000 Genomes Project [https://www.internationalgenome.org/], gnomAD (genome aggregation database) [https://gnomad.broadinstitute.org/]) or in the scientific literature and ClinVar [https://www.ncbi.nlm.nih.gov/clinvar/]3) evolutionary conservation of the affected nucleotide (PhyloP [6] score or GERP [7]) and pathogenic potential by in silico predictive alghorithms (SIFT [8], PolyPhen-2 [9], MutationTaster [1–21], Align GVGD [11]).

Classification was performed according to ACMG2015 guidelines [12] by Intervar [http://wintervar.wglab.org/] and Cardio Classifier tools [https://www.cardioclassifier.org/index.php]. All putative sequence variants found by NGS were validated by Sanger sequencing.

3. Results and discussion

Case1: From the NGS analysis, SCN5A (NM_198056.2) c. 611 + 1 G > A, p.(?) was detected in heterozygous status.This variant is located in the donor splice site of intron 5, the consequence of this change is not predictable but the skip of exon 5 is very likely. The identified variant is described in the literature [13] and it is annotated in ClinVar as pathogenic/likely pathogenic (ClinVar Variation ID:201,427); according to ACMG2015 recommendations for variant interpretation, it is at present classifiable as pathogenic (PVS1, PM2, PP3, PP5). The genotype is consistent with a form of chanellopathy.

Following the result of the NGS analysis in the deceased proband, the genetic test was performed in her son with a positive result.

Case2: From the NGS analysis, MYH7 (NM_000257.3) c.5287 G > A, p.(Ala1763Thr) was detected in heterozygous status. This variant is located in exon 37; the alanine residue is highly conserved and there is a small physicochemical difference between alanine and threonine. This variant has been reported in individuals with hypertrophic cardiomyopathy [14–17], one individual with dilated cardiomyopathy [18] and individuals affected with sudden unexplained death [19] and it is annotated in ClinVar as Uncertain significance (ClinVar Variation ID:1,778,469). According to ACMG2015 recommendations for variant interpretation specifically adapted for MYH7-associated inherited cardiomyopathies [20], the identified variant is at present classifiable as probably pathogenic (PS4, PM2, PP3). The genotype could be compatible with the autopsy diagnosis of hypertrophic cardiomyopathy. Genetic test in proband's sister is in progress.

4. Conclusion

NGS analysis of a large panel of genes related to hereditary arrhythmias and cardiomyopathies should be included in forensic analysis protocols for cases of sudden death and represents a kind of continuation of the autoptic examination, previously defined as molecular autopsy [21].

This approach could have a great utility in the diagnostic definition of a clinical phenotype when the cause of death is non clear and it could serve as a definitive confirmation test in presence of clinical indication.

In both cases genetic analysis is essential for the early screening of at-risk family members and for their inclusion in clinical care pathways.

The analysis of multiple genes improves sensitivity in comparison with Sanger sequencing but it complicates the analytical approach; correct interpretation of NGS data is essential to get highly specific results then segregation analysis can be fundamental for the correct definition of variants of uncertain clinical significance.

Declaration of Competing Interest

None.

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