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Case Report: Molecular Complement to the Medico-Legal Autopsy in a Case of Unexplained Death

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Abstract: *The determination of sudden cardiac death during a medical-legal autopsy is a challenging process, particularly in the absence of a prior clinical record of structural alterations, both macroscopically and microscopically. This makes it difficult to diagnose. We present a case in which a molecular analysis was conducted using parallel sequencing of a new generation. This analysis revealed the presence of potentially pathogenic variants in the PRKAG2, SCN4B and LDLR genes, which are associated with sudden cardiac death.*

Keywords: *Molecular diagnosis, sudden cardiac death, cardiomyopathies and channelopathies cardiac*

1. INTRODUCTION

Sudden death (SD) is considered a natural death, occurring rapidly and unexpectedly in individuals who appear healthy. It may occur within a short interval of one to six hours or instantly due to the emergence of symptoms [1].

Sudden cardiac death (SCD) occurs within the first hour of the onset of symptoms. Patients found dead within 24 hours of being asymptomatic are presumed to have died of cardiac arrhythmia or hemodynamic catastrophe [2].

According to a report by the American Heart Association, there were approximately 356,000 out-of-hospital cardiac arrests in the United States in 2022, of which 90% were fatal, and the survival rate for those discharged from the hospital was 10% [3].

It has been reported that the causes of SCD of structural origin vary in the different age sections. For older people, coronary artery disease and cardiomyopathies account for more than 85% of cases, with the most common being hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular myopathy (ARVC), restrictive cardiomyopathy (RCM), dilated cardiomyopathy (MCD), and non compaction of the left ventricle (LVNC). In contrast, under 35 years of age represent 40% of the total population.

The results indicate that cardiomyopathies can occur in the absence of a diagnostic phenotype prior to the development of SCD. Additionally, the presence of minimal structural changes at autopsy should not preclude the consideration of canalopathies. [4,5].

The genetic component of these diseases encompasses a broad range of established causal genes, probable causal genes, and genes associated with the disease. The heritability of these heart diseases caused by common variants and rare variants is estimated to range between 38% and 50% based on multiple studies [5,6].

SCD by arrhythmogenic mechanisms (primary electrical disorders), which presents in individuals under 40 years of age, is estimated to have a prevalence of approximately 5 in 10,000. These disorders are hereditary and exhibit incomplete penetrance; they can potentially result in lethal arrhythmias [7,8].

The most common are long QT syndrome (LQTS), short QT syndrome (SQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT) [6,7,8]. These syndromes are distinguished by

specific electrocardiogram (ECG) abnormalities. The different types and locations of pathogenic variants are highly correlated with the severity of the phenotype, which affects the severity of the disease in these syndromes [9].

Since the identification of these mutations is challenging, novel methodologies are being developed to facilitate this process. One approach, next-generation sequencing (NGS), has been employed to elucidate the genetic factors of complex disorders, including cancer, congenital malformations, and neuro developmental conditions such as autism spectrum disorder (ASD) [10,11].

This is one of the reasons for the increased number of cases analysed by NGS. The number of genes associated with SCD has increased from 194 to 380. The disadvantage of this is that the number of variants of uncertain significance has increased, too. Consequently, the authors regularly recommend the classification of these variants. For further information on this subject, please see references 12 to 14.

A post-mortem molecular analysis is required to determine the potential pathogenicity of variants observed in genes such as RYR2, CACNA1C, ANK2, MYH7, LDB3, PKP2, JUP, DSG2, DSP, TMEM43, TBX5, and GATA4. To achieve this goal, an in-silico analysis should be employed to ascertain the degree of pathogenicity of these variants [15, 16].

2. Clinical Presentation

A summary of the pertinent facts is as follows: A teenage male was discovered by his mother in a state of unconsciousness in the bed of the room. He was subsequently transported to a medical facility, where he was admitted without vital signs.

The autopsy revealed the following findings: The adolescent male exhibited no evidence of significant trauma. However, nonspecific findings of hypoxia were observed. Consequently, samples of different anatomical components were retained for complementary histology and toxicology studies. The underlying cause of death is as follows: The case is currently under study. The manner of death is as follows: The cause of death is currently under investigation.

The pericardium exhibited a smooth surface with normal-appearing cavities. The right ventricular wall was 4 mm in thickness, while the left ventricular wall was 10 mm and the septum was 10 mm. The dimensions of the tricuspid valve, pulmonary valve, mitral valve, and aortic valve were 10 cm, 8 cm, 10 cm, and 7 cm, respectively. No evidence of traumatic injury or dissection was observed.

The coronary arteries exhibited an anatomical configuration that was consistent with a normal appearance. They were dissected through multiple transverse sections and no emboli were observed. The coronary ostia were also found to be permeable.

Aorta and Large Vessels: No evidence of traumatic injury or obstruction was observed.

The veins exhibited no evidence of traumatic injury and were found to be permeable to the cut.

The toxicological study yielded negative results for ethanol and methanol, as well as for psychoactive substances, benzodiazepines, phenothiazines, antidepressants, amphetamines, Trazodone, Sildenafil, Zolpidem, Clozapine, Scopolamine, Ketamine, Norketamine, and Haloperidol.

A histopathological study revealed the following diagnoses: pulmonary hemorrhage, pulmonary edema, anthracosis, hypoxic neuronal injury, acute splenic congestion, hepatic acute passive congestion, adrenal stress, and generalized visceral congestion.

3. Methodology

The extraction and quantification of DNA were conducted in accordance with the following protocol:

The isolation of DNA from blood was accomplished through the utilization of the QIAamp kit for DNA Blood Midi/Maxi, in accordance with the specifications outlined by the manufacturer.

The quantification of DNA and libraries was conducted using the Quantifying dsDNA Kit on the Quantus. The fluorometer instrument was utilized in accordance with the manufacturer's instructions.

The TruSight Cardio-Enrichment kit was utilized in accordance with the manufacturer's instructions.

Directed sequencing was performed using the Illumina MiSeq system (Illumina Inc., San Diego, CA, USA) with the MiSeq Reagent V3

(150 cycles) according to the manufacturer's instructions. Sequence analysis and variant filtering were conducted using the proprietary MiSeq Analysis Viewer software (Illumina Inc., San Diego, CA, USA).

The exome variants were annotated through the integration of SnpEff and ANNOVAR. The selection of variants was guided by the recommendations of the European Heart Rhythm Association (EHRA) 2022 and American College of Cardiology (AHA/ACC) 2020.

4. Results and Discussion

Molecular analysis revealed the following results: The PRKAG2 gene variant, rs76351165, has been associated with pathology including Wolff Parkinson's syndrome and glycogen storage disease of familial hypertrophic cardiomyopathy.

The SCN4B gene variant, rs117263855, is associated with Long QT Syndrome 1, also known as Roman Ward syndrome, and the LDLR gene variant, rs200793488, is linked to familial hypercholesterolemia.

Table 1. *Variants detected with molecular diagnosis in the case of unexplained death*

Gen	Funtion	Sequencechange	Variant	Patology	Clinicalsignificance
PRKAG2	UTR5	NM_0116203: c-90G>T	rs76351165	Wolff-Parkinson-White pattern, Hypertrophic cardiomyopathy 6	Benign, Likely-Benign
SCN4B	UTR3	NM_001142349.1:c.*1071T>C,	rs117263855	Congenital long QT síndrome, Romano-Ward Syndrome	likely-benign
LDLR	EXONIC	p.Arg744*	rs200793488	Hiper	Pathogenic

Genetic variants should be correlated with findings from legal medical autopsy and ante mortem clinical records if any.

Taking into account the results, the death is explained by cardiac rhythm disorder due to sudden cardiac death, which is due to genetic variants associated with sudden cardiac death, all of which are hereditary, most likely because of how it manifested it may correspond to an arrhythmogenic canalopathy.

It is important to note that the variant rs76351165 is correlated with the Wolff-Parkinson-White (WPW) syndrome. This is a congenital cardiac abnormality that presents clinically as paroxysmal episodes of palpitations, which, though potentially concerning, are generally benign in nature. Nevertheless, the probability of sudden cardiac death in these patients is low, with the exception of certain cases where it has been documented to be elevated [17]. Additionally, it has been demonstrated that the variant is also associated with glucogenosis, a condition resulting from the alterations (mutations) in one of multiple genes.

The condition is inherited in an autosomal recessive manner. It is known that the majority of hepatic glucogenoses manifest similar symptoms, although the degree of severity varies between different types of diseases, resulting in symptoms that typically manifest during early childhood. A lack of enzyme function results in hepatomegaly, hypoglycemia, elevated blood cholesterol levels, and stunted growth. Children may be perceived as clumsy and may tire more quickly during physical activity.Furthermore, the variant rs76351165 affects the heart muscle (myocardium), which can lead to serious heart disease [18].

Familial hypertrophic cardiomyopathy is the autosomal dominant inherited form of hypertrophic cardiomyopathy. In some cases, the initial symptom of this disease can manifest as sudden death, particularly in individuals between the ages of 14 and 35 and during intense physical exertion during sports activities [19].

In contrast, rs117263855 is associated with Long QT Syndrome (LQTS), which is a type of arrhythmogenic canalopathy. It is characterized by a significant alteration in ventricular repolarization, which is electrocardiographically translated by an extension of the QT interval. This predisposes to sudden death by malignant ventricular arrhythmias. At least 10% of patients with affected LQTS present with sudden death as their initial symptom and, in some cases, as their terminal one. The syndrome may present with syncope, cardiac arrest, or sudden death during activities such as exercise and running. In addition, sudden death may result from

intense emotional stimuli, such as during an argument. Sudden death may also occur in situations such as hearing the alarm sound of the clock or phone ringing. Furthermore, sudden death may occur during sleep and during periods of slow heart rate [20].

In the case of Romano-Ward syndrome, it is a form of familial long QT syndrome (LQTS) that is characterized by syncopal episodes and electrocardiographic abnormalities, including prolongation of the QT interval, T wave anomalies, and ventricular tachycardia type torsion of the tips (TdP). Most patients develop symptoms during exercise or in response to stress or emotional disturbances, and symptoms rarely occur at rest or during sleep [21].

Finally, variant rs200793488 is associated with Familial Hypercholesterolemia (FH), an inherited disease that manifests from birth. FH is characterized by elevated plasma cholesterol levels, predominantly in the form of low-density lipoproteins (c-LDL).

It is recommended that those genetic variants be correlated with findings from legal medical autopsy and antemortem clinical records, if any, to gain a more comprehensive understanding of the case. In consideration of our findings, it is proposed that the death can be attributed to a cardiac rhythm disorder resulting in sudden cardiac death, which is associated with genetic variants predisposing to sudden cardiac death. These variants are hereditary in nature. It is probable that the manner in which the disorder manifested corresponds to an arrhythmogenic channelopathy.

5. Conclusion

In the present case, the cause of death was determined to be sudden death of cardiovascular origin secondary to genetic variants. The mode of death was classified as natural.

The probable diagnosis was based on the use of new approaches to support classical autopsy studies. The implementation of molecular autopsy, as presented here, is of great importance in order to be able to respond to the cause of death in those cases classified as undetermined death by conventional autopsy.

6. Limitations of the Study

The inability to perform comprehensive functional testing on all candidate variants and to corroborate the pathogenicity results obtained in silico through cosegregation studies with relatives.

7. Ethical Considerations

The ethical standards set forth in the revised Declaration of Helsinki were observed, in addition to the relevant legislation, decrees, and resolutions governing viscerotomies and the utilization of forensic materials for academic and research purposes at the National Institute of Legal Medicine and Forensic Sciences.

8. Declaration of Competing Interest

It is the authors' intention to state that they do not possess any competing financial interests or personal relationships that could have appeared, at least to some extent, to influence the work reported in this paper.

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