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Targeted next generation sequencing application in cardiac channelopathies: Analysis of a cohort of autopsy-negative sudden unexplained deaths

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ABSTRACT

Genetic testing for cardiac channelopathies in sudden unexplained death (SUD) has developed substantially over the last years. The Next Generation Sequencing (NGS) technology provides an unprecedented opportunity to screen for genetic variations underlying arrhythmogenic genes in a short period of time at a low cost.

The present study aimed to perform genetic testing with NGS technologies on the Ion Torrent Personal Genome Machine™ (Ion PGM™) sequencer, in targeting a total of 23 genes reported to be associated with inherited cardiac channelopathies in order to identify the possible cause of death in a cohort of post-mortem cases.

The molecular analyses focused on 16 cases of SUD, aged less than 35 years old. In all cases, the cause of death could not be determined after a rigorous autopsy associated with histopathological and toxicological analyses according to the guidelines of the Association for European Cardiovascular Pathology. DNA was extracted from fresh frozen tissue.

An average of 200 variants was identified per case. However, after the prioritization process using a new scoring program (VaRank) and after the conjunction of clinical data and molecular findings, four "likely pathogenic" variants (including two undescribed variants), were identified in three cases (18.75%) of our cohort in the genes *KCNH2*, *ANK2*, *SCN5A* and *RYR2*. One case, who died during psychiatric hospitalization after administration of a QT prolonging drug, showed a double "likely pathogenic" variant in Long QT genes (*ANK2* and *SCN5A*) which may have predisposed to drug-induced cardiac arrhythmias.

Our study illustrates that the NGS approach based on AmpliSeq™ libraries and Ion Torrent PGM™ sequencing may be an efficient approach, integrated to post-mortem examination. Given the massive amount of information generated by NGS, a rigorous filtration strategy of variants coupled with multidisciplinary collaboration is crucial to determine the potential pathogenic role of identified variants in the cause of death.

1. Introduction

In developed countries, sudden cardiac death (SCD) is considered one of the most common causes of death, representing a major health problem [1,2]. Although the majority of cardiac death victims are elderly, many children and young adults under the age of 35 years die each year due to various cardiac pathologies. Structural cardiovascular abnormalities are often evident at autopsy, including hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy (ARVD), myocarditis or congenital coronary artery anomalies [3,4]. However, a review of 5 population-based investigations of sudden death in young people by Tester and Ackerman [5] showed that nearly 30% of SCDs in

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young people are autopsy-negative. These cases are called sudden unexplained deaths (SUD). Many of the SUD cases are suspected to be caused by inherited cardiac channelopathies such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPTV), Brugada syndrome or short QT syndrome (SQTS). In recent years, pathogenic mutations have been identified in several genes related with the cardiac channelopathies [6,7].

Different technologies and genetic approaches have been developed for the diagnosis of these cardiac disorders. Some of them enabled mutation targeting, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [8,9], or the SNaPshot technology [10] whereas others allowed for the scanning of known genes notably with the High Resolution Melting (HRM) analysis [11–13] or the searching for new genes like Genome-Wide Association Studies (GWAS) [14].

Because formalin fixed paraffin embedded tissues (FFPE) samples were initially, at least in France, the principal source of DNA in the problematic of SUD and because mutation detection by classical serial molecular techniques, like direct sequencing, was time consuming and expensive, we previously evaluated two different methods in a cohort of SUD with a comparison between results derived from fresh frozen and FFPE tissue [11]. HRM analyses were first performed on the *KCNQ1* gene which was selected for its low number of small exons and because it represents a large percentage of the long QT syndrome mutations (40–-55% according to Hedley et al. [15]). MALDI-TOF MS was then performed on the *RYR2* gene, a very large gene that hampered classical genetic investigations. Indeed only "three hot spot regions" (representing 1858 amino acids out of 4967 amino acids) were screened [16,17]. No variants with amino acid changes and therefore with potential functional effect on ion channels were detected in this initial study. The poor diagnostic yield in our cohort using this strategy prompted us to complement the screening with the other genes involved in channelopathies.

In recent years, frozen sample collection from SUD cases has been considerably improved in France and facilitated post-mortem genetic investigations. In parallel, the advent of next generation sequencing (NGS) technologies opened new perspectives in the post-mortem diagnosis of cardiac channelopathies and provided unprecedented sequencing capacity at dramatically lower costs.

In the literature, the efficiency of NGS based approaches has already been described in patients with channelopathies, mainly to sequence LQTS gene panels. The systems used were the SOLID™ system from Life Technologies, the MiSeq system from Illumina or the Ion Personal Genome Machine™ (Ion PGM ™) from Life Technologies [18–20]. Until now, to our knowledge, only three studies have performed genetic testing using NGS technology on post-mortem samples of SUD. The study of Brion et al. [21] used the SOLID™ system and the studies of Hertz et al. [22] and Campuzano et al. [23] used the MiSeq Illumina sequencer.

Therefore the aim of our investigation was to perform genetic testing using NGS technologies in a cohort of autopsy negative SUDs in order to identify genetic variants that could play a role in the mechanism of sudden death. We sequenced a total of 23 genes reported at the time of panel construction to be associated in the literature with inherited cardiac channelopathies.

2. Materials and methods

2.1. Investigation process

During 5 years (2008–2012), fresh heart (right ventricle, left ventricle, septum) and liver tissue was retained and frozen at −80°C at the initial autopsy of individuals 0–35 years of age, where the cause of sudden death was not immediately apparent. After 4–6 weeks, after which time the histopathology and toxicology results became available, we determined if genetic investigations should start within the judicial mandate which is to determine the cause of death. The inclusion criteria were: no organic abnormality, no macroscopical or microscopical abnormality of the heart and no specific finding during the toxicological investigations. All the histopathological examinations of the heart were realized by the same histopathologist specialized in cardiac diseases according to the guidelines of the Association for European Cardiovascular Pathology [1].

2.2. Sample selection

Samples were stored at the time of autopsy from 42 unrelated sudden unexplained deaths in young victims. In 26 cases, laboratory tests revealed a cause of death such that molecular autopsy was not required. These included 8 cardiac diagnoses such as acute myocarditis (3), acute myocardial infarction (4) or endocardial fibroelastosis (1) and 18 various non-cardiac diagnoses including respiratory infections (3), pulmonary embolism (2) and narcotic or drug intoxication (13). Sixteen cases were referred for channelopathy gene screening: 8 males and 8 females, with a median age of 10.61 years old (range 8 days–34 years). The circumstances of the deaths were provided by police records. The clinical characteristics of the cohort are presented in Table 1. Sixteen cases were included in the cohort following the above mentioned criteria.

2.3. DNA extraction and quantification

DNA was extracted from the frozen samples and purified with the QIAamp DNA Mini® Kit (Qiagen) according to the manufacturer's instructions. Quantification of the DNA samples was performed using the NanoDrop™ 8000 Spectrophotometer (Thermo Scientific®).

2.4. Targeted capture and massively parallel sequencing

A total of 23 genes, reported to be associated with inherited cardiac channelopathies in the literature at the time of the panel

Table 1
Characteristics of the cohort.

Case number	Gender	Age	Circumstances of death
1	M	20 yrs	During physical activity
2	M	7 m	Discovered in side sleep position by his mother
3	M	20 yrs	Discovered in his bathroom
4	F	34 yrs	During sleep, antecedents of epilepsy
5	F	20 yrs	During a psychiatric hospitalization, patient under neuroleptic therapy, antecedents of autism spectrum disorders
6	M	26 yrs	While on ride in an amusement park
7	F	4 m	During sleep
8	M	2 m	During sleep in side sleep position
9	F	3 yrs	During sleep
10	F	16 yrs	Sensation of cardiac palpitations preceding a syncope
11	F	8 d	During sleep
12	M	3 m	During sleep
13	F	3 yrs	During sleep
14	M	2 yrs	Bout of vomiting without dehydration symptoms
15	F	6 m	During sleep
16	M	24 yrs	During sleep in prison, antecedents of epilepsy

F, female; M, male; d, days; m, months; yrs, years.