G81 Detection of KCNQ1 Genetic Variations by High Resolution Melting Analysis for the Diagnosis of Channelopathies in Postmortem Investigations

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After attending this presentation, attendees will be informed of the great interest of the high resolution melting method used for genetic variations screening in cardiac ion channel genes in postmortem investigations.

This presentation will impact the forensic science community by demonstrating the application of a recently developed molecular technique, high resolution melting (HRM), for the detection of genetic variant on genes implicated in channelopathies in postmortem investigations.

In developed countries, sudden cardiac death (SCD) is one of the most common causes of death. One of the largest epidemiological studies of unexpected deaths in young people showed that more than half of the deaths were of cardiac origin and in 29% no recognizable cause was identified at postmortem (Tester et al., 2007).[1]

Potentially lethal ion channel disorders (channelopathies) such as long QT syndromes, catecholaminergic polymorphic ventricular tachycardia (CPTV) and the Brugada Syndrome may be responsible for a portion of such cases of sudden death in young persons.

Postmortem genetic testing for sequence variations in cardiac ion channel genes has become an important tool for elucidating the cause of sudden cardiac death (Ackerman et al, 2001; Kauferstein et al., 2009).[2] Formalin-fixed and paraffin-embedded tissue (FF-PET) as well as frozen tissue could be used as source of DNA in postmortem investigations. If frozen tissue is undoubtedly the greatest source of intact DNA, in some cases FF-PET is the unique source of genetic material.

In this context, the purpose of our study was first to validate a successful DNA extraction and purification method corresponding to the association of phenol-chloroform extraction and silica-based purification protocols. This protocol was previously reported in ancient DNA studies on archaeological bones but had not been used for DNA extraction from FF-PET. The second step consisted of genetic investigations on frozen and FF-PE tissues in each case of sudden death involving adult younger than thirty-five years with no significant morphological anomalies particularly with no cardiac structural disease and with negatives toxicological investigations. The samples studied were collected from autopsy cases performed at the Institute of legal Medicine from Strasbourg (France). The autopsy practice and modalities of sampling were realized according to the recommendations of the "European Cardiovascular pathology Association" (Basso et al. 2008).[3] The KCNQ1 gene was chosen in a first approach.

Since, according to the literature, mutations on this gene are randomly distributed, genetic screening was performed for each studied case, with the HRM method on the LightCycler 480 (Roche). The HRM is a technique that can detect sequence changes in amplicon through monitoring of the fluorescence of a double DNA binding dye which dissociates from DNA as it denatures with increasing temperature. If sequence changes are present within the amplicon, they cause a difference in the melting profile compared with wild-type. The principle of this methodology will be more developed in the presentation.

The comparison of results obtained with frozen and FF-PE samples showed that the two types of samples have a great interest in the genetic investigations. The advantages and limits of each type of samples will be discussed in details. From this study, it appeared that the HRM is a rapid, cost-effective and specific method allowing identification of KCNQ1 genetic variations and avoids systematic sequencing of the entire coding region of gene of interest in postmortem investigation of sudden cardiac death.

References:

1 Tester DJ, Ackerman MJ. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. *J Am Coll Cardiol*, 2007, 49, 240-6.

2 Kauferstein S, Kiehne N, Neumann T, Pitschner HF, Bratzke H. Cardiac gene defects can cause sudden cardiac death in young people. *Dtsch Arztebl Int*. 2009;106(4):41-7

3 Basso C, Burke M, Fornes P, Gallagher PJ, de Gouveia RH, Sheppard M, Thiene G, van der Wal A; on behalf of the Association for European Cardiovascular Pathology. Guidelines for autopsy investigation of sudden cardiac death. Virchows Arch. Jan, 2008, 452(1):11-8.

High Resolution Melting, KCNQ1, Formalin-Fixed and Paraffin- Embedded Tissue

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