Consideration of molecular autopsies in forensic cases of sudden unexpected death in infants and children in South Africa

Fatalities that are sudden and unexpected are referred for medicolegal investigation under the National Health Act No. 61 of 2003 in South Africa (SA).[1] Post-mortem investigation of these cases may include an autopsy, death scene investigation and clinical history review, which are usually sufficient to determine cause of death. However, in some instances, the cause of death remains undetermined.[2]

A retrospective study at Tygerberg Forensic Pathology Services (Cape Town) between 2001 and 2005 reported that sudden unexpected death (SUD) comprised 6.1% (*n*=826) of the total caseload (*N*=13 536), and of these cases, 8.8% (*n*=72) remained undetermined after post-mortem investigation.[2] This was more than double the proportion of undetermined SUD cases reported in England (4.1%; *n*=23) during a similar time period.[3] Further, the number of infants (<1 year of age) who die suddenly and unexpectedly represents an unusually high burden of mortality in SA. A multicentre study in SA estimated that 8.7% of sudden unexpected infant death cases admitted for forensic investigation remained as having an 'undetermined' cause of death.[4]

The use of genetic analysis is becoming increasingly relevant, particularly in research settings, to investigate cases that remain undetermined following post-mortem investigation.[5-8] The analysis of DNA from a deceased individual with the aim of identifying genetic variants associated with or causative of a fatal disease (particularly those with a sudden onset) is referred to as a molecular autopsy. The genetic variants targeted during a molecular autopsy are most often associated with diseases that do not manifest with obvious and specific pathological changes.[9] Examples include some variants in *KCNQ1* and *SCN5A* that are causative of suboptimal electrical functioning of the heart (e.g. Brugada syndrome),[10] as well as variants associated with metabolic disorders that are often masked by signs of mild infection at autopsy.[5,11] Hence it is unlikely that these genetic-based diseases would be detected during a conventional medicolegal autopsy.

The value of a diagnosis in an otherwise undetermined case is that it brings closure to the family that has suffered loss, and this accurate mortality data has positive implications for both the legal and public-health sectors.[12] Some studies have also demonstrated the clinical value of a forensic genetic analysis, when reportable genetic mutations were relayed back to blood relatives.[13,14] These individuals were then linked with appropriate clinical and genetic screening to better understand and/or manage their own risk of possibly having the same disease that caused the death of their relative. In the case of infant deaths, parents may find this genetic information valuable for future pregnancy management.

Studies have shown varying degrees of resolution of previously undetermined cases following a molecular autopsy, with Anderson *et al.*[15] demonstrating up to 44% resolution in an SUD cohort aged 1 - 19 years. The successful resolution reported in various studies appears to be dependent on numerous variables, such as the age of the cohort, selection criteria of the cohort and the genes targeted for genetic analysis.[10,16,17] The potential resolution of cases, combined with the value for the family, have made molecular autopsies an attractive prospect in investigations of SUD.

It is important to note that there is no single 'sudden death gene',[18] and therefore finding a plausible genetic variant that might have contributed to sudden death may require repeated genetic testing. This approach therefore requires the availability of a biological sample that yields sufficient quantity and quality of DNA for multiple tests. Retrospectively speaking, tissue may have been collected from SUD cases at forensic autopsy for histopathology analysis, and stored. Tissue is typically formalin-fixed and paraffin-embedded to preserve cell morphology; however, this poses significant difficulties in the amount and quality of usable DNA that can subsequently be retrieved for a molecular autopsy.[19]

If collecting samples prospectively, 20 mL of blood is the recommended sample for post-mortem genetic testing.[20] Realistically, this volume of blood is exceptionally challenging to obtain from deceased infants, as post-mortem changes, including clotting, increase the viscosity of the blood. However, if an internal autopsy is necessary to determine cause of death, a sufficient volume of blood can be obtained from infants (e.g. heart blood) for a molecular autopsy. In our experience researching molecular autopsies on cases at Salt River Mortuary, Cape Town, even 1 mL of blood has sufficed (yielding a median of 103 µg of DNA).

A massively parallel sequencing approach, which requires less DNA (as, technically, only one test is performed), may also be used to analyse many genes simultaneously. This method requires complex instrumentation and highly skilled personnel, neither of which are routinely found in forensic pathology settings. As such, collaboration between fields is of utmost importance. While the cost of processing samples using massively parallel sequencing has decreased, and is anticipated to decrease further, the limiting factor is still the time required to analyse and interpret the genetic results, whereby the more genes analysed, the more time required for interpretation.

The value of performing molecular autopsies to resolve forensic cases of SUD that would otherwise have remained undetermined is well documented. Its application has been demonstrated in other countries, yet research into this important area of forensic genetics is currently lacking in SA.[17] To this end, the University of Cape Town and Salt River Mortuary have engaged in collaborative research to investigate SUD in Cape Town and the applicability of molecular autopsies in a local setting. We urge other academic and forensic professionals to also engage in the recruitment of statistically powered cohorts of unexplained cases of SUD (particularly of infants and children), possibly also biobanking blood samples for future genetic analysis. Guidance pertaining to the ethical, legal and logistical challenges that accompany such research is provided by Heathfield *et al.*[21] Together we can strive towards better and more integrated healthcare, even after death.

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