The added value of molecular-based diagnostics in the African forensic medical setting

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Abstract

Sudden unexpected infant death (SUDI) is reported to be an extraordinarily high burden in sub-Saharan Africa, with the incidence rate in South Africa among the highest in the world. It is common for the cause of many such infant deaths to remain unexplained even after a full medico-legal death investigation, and then to be categorised as a sudden unexplained infant death (SUID). Fortunately, advances in molecular-based diagnostics allow researchers to identify numerous underlying inherited cardiac arrhythmogenic disorders in many SUDI cases, with a predominance of variants identified in the *SCN5A* gene. Such cardiac arrhythmogenic-related sudden deaths generally present with no structural alterations of the heart that are macroscopically identifiable at autopsy, therefore highlighting the importance of post mortem genetic testing. We report on a significant genetic finding that was made on a SUDI case in which the cause was ascribed to an acute bacterial pneumonia but it was still subjected to post mortem genetic testing of the *SCN5A* gene. The literature shows that many SUDI cases diagnosed with inherited cardiac arrhythmogenic disorders have demonstrated a viral prodrome within days of their death. It is therefore not uncommon for these cardiac disorders in infants to be mistaken for flu, viral upper respiratory tract infection or pneumonia, and without the incorporation of post mortem genetic testing, any other contributory causes of these deaths are often disregarded. This study highlights the need for research reporting on the genetics of inherited cardiac disorders in Africa.

Keywords: channelopathies, dilated cardiomyopathy (DCM), inherited cardiac disorders, post mortem genetic testing, *SCN5A*, sudden unexpected death in an infant (SUDI).

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Sudden deaths in infants are still considered one of the leading causes of infant mortality worldwide and have also been reported to be an extraordinarily high burden in sub-Saharan Africa (SSA).[1-3] According to Duncan *et al*.,[4] for most countries, the rate of sudden unexplained infant deaths (SUIDs) [or the previously termed sudden infant death syndrome (SIDS) cases] is reported at approximately 0.2–0.5 per 1 000 live births. The most recent published incidence rate for South African SUID cases was 1.06 per 1 000 live births for the white population and 3.41 for infants from the mixed-ancestry population group, respectively.[2]

The investigation into SUDIs and child mortality remains a high-priority research area in South Africa.[2-3,5] It has universally been accepted that a SUID case can very rarely be explained by a convenient and simplistic 'single-cause' mechanism, but instead is attributed to a complex event with an increase in incidence when risk factors such as vulnerability, a critical period in development and exogenous stressors all intersect at the same time (triple-risk model proposed by Filiano and Kinney).[4,6,7]

One of these risk factors, and a possible preventable cause of SUIDs, which has received increased attention over the past few years is inheritable cardiac arrhythmogenic disorders.[1,3,4] Although these inherited cardiac disorders in SUID cases have primarily been associated with electrical conditions (channelopathies), recent studies have identified variants in genes encoding structural proteins, thereby suggesting a cardiomyopathy as a possible cause of death as well.[1,8-10]

Previous studies demonstrated a link between SUIDs and a predominance of *SCN5A* gene variants. This could be explained by the known genotype–phenotype correlations that suggest patients with *SCN5A* variants may experience a higher mortality rate, mostly occurring during sleep, compared to patients suffering from variants in other genes involved in inherited cardiac diseases.[1,8]

Advances in molecular-based diagnostics allow researchers to identify numerous underlying inherited cardiac arrhythmogenic disorders that have been misdiagnosed in many SUID cases.[1,11] In many developing countries, including Africa, there is still a significant lack as far as forensic molecular diagnostics is concerned, mainly due to financial and resource constraints.[12] As a result, the cases in this study were subjected to retrospective post mortem molecular analysis of only the most prevalent gene (*SCN5A*) associated with SUID, in order to identify any possible pathogenic variations associated with an inherited cardiac disease, which may have predisposed this infant to a sudden death.

Case report

We report on a case of a two-month-old male infant of African ancestry whose mother found him unresponsive in his crib during a scheduled nap. Upon emergency medical services (EMS) arrival, the infant was declared dead at the scene without

any medical care being administered. No written clinical history/records were available, however his mother reported him having a recent cold for which she administered cough medication. The mother also reported an increase in crying and that the infant struggled to feed.

Due to the sudden and unexpected nature surrounding the death, the body was admitted to the Pretoria Medico-Legal Laboratory for further medico-legal investigation, in accordance with the Inquests Act 58 of 1959. A complete macroscopic autopsy examination was conducted, which externally revealed the deceased to be of average physique and nutritional state. No injuries were noted on the body.

Upon internal examination, the intracranial examination showed no gross pathological changes. Examination of the heart revealed no abnormalities involving the epicardium. The myocardium and heart valves appeared normal. The lungs appeared congested and oedematous. On cut surfaces, the lungs showed sharply defined edges, had a friable texture and contained muco-purulent fluid. Examination of the stomach revealed contents of a milk-like residue and it was noted that the gastric mucosa appeared normal. As a result, no macroscopic cause of death could be identified at autopsy.

Toxicology results revealed only trace amounts of theophylline, a bronchodilator, which is in keeping with the history of cough medication administered to the infant. No sedatives could be detected in the blood specimen.

Histological examination of the thymus, brain and heart showed no obvious pathological changes. Sections of the heart showed no evidence of myocyte hypertrophy, nucleomegally or interstitial fibrosis. Sections of the lungs showed a mild mononuclear interstitial infiltrate with thickening, congestion and focal haemorrhage (Fig. 1). Focal intra-alveolar neutrophilic exudate was also noted in the lungs, as seen in Fig. 2. The features noted in the lungs were found to be in keeping with an acute bacterial pneumonia. Henceforth, the primary medical cause of death was acute bacterial pneumonia.

Genetic testing

Due to the diagnosis of an acute bacterial pneumonia as the cause of death, this infant case study was included in our larger study. For this case, an archived, formalin-fixed, paraffin-embedded (FFPE) myocardial tissue sample, obtained from the original autopsy 10 years prior to this study, for histology purposes, was subsequently subjected to retrospective post mortem genetic testing of the *SCN5A* gene. DNA was extracted from the FFPE myocardial tissue sample using the QIAamp DNA FFPE tissue kit (Qiagen). After extraction, the concentration and purity of the DNA sample was determined spectrophotometrically (NanoDrop spectrophotometer, Thermo Scientific).

Thirty-nine primer pairs were used for amplification of 28 exons of the *SCN5A* gene.[13] High-resolution melt real-time polymerase chain reaction (PCR) amplifications were performed using SensiFast HRM mastermix (Bioline) on the RotorGene Q (Qiagen). Following optimisation, DNA concentrations used per reaction averaged 50 to 80 ng. Final primer concentrations were 10 pmol.

Thermal cycling conditions followed SensiFast guidelines, and annealing temperatures were dictated by the primers. High-resolution melting (HRM) analysis was performed, and control and case study samples were compared. All amplicons that showed variation on HRM were subjected to sequencing (Inqaba Biotec). Sequencing results were analysed using CLC Main Workbench 5 software (CLC Bio®) and were aligned with the *SCN5A* gene sequences from GenBank (SCN5A NG_008934.1; NM_001160161.1 and NP_001092874.1) [National Center of Biotechnology and Information (NCBI)]. Polymorphism phenotyping v2 (PolyPhen2) was used to determine the probability of pathogenicity for novel identified variations.

Results

Genetic analysis revealed two different variations in exon 28 of the *SCN5A* gene. The first was a novel heterozygous variation (c.5566G>A) in the coding DNA sequence. This missense variation leads to a G>A nucleotide change in codon 1856, with an amino acid change of alanine (Ala) to threonine (Thr) (p.A1856T) (Fig. 3). Due to the functional difference between these two amino acids, the possibility of this variation affecting the protein structure is high. The PolyPhen-2 online algorithm predicted this variant to be probably damaging with a score of 1.000.

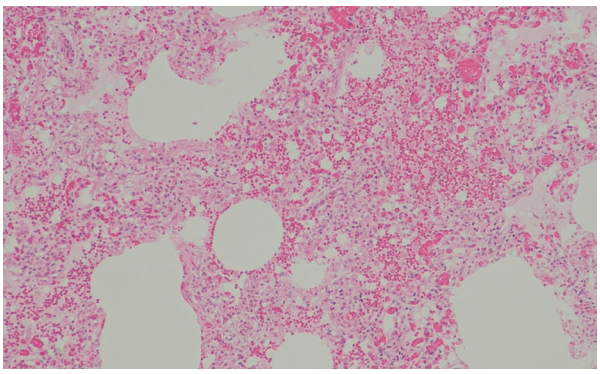

Fig. 1. Haematoxylin and eosin stain of the lungs tissue shows a mild mononuclear interstitial infiltrate with thickening, congestion and fresh focal haemorrhage.

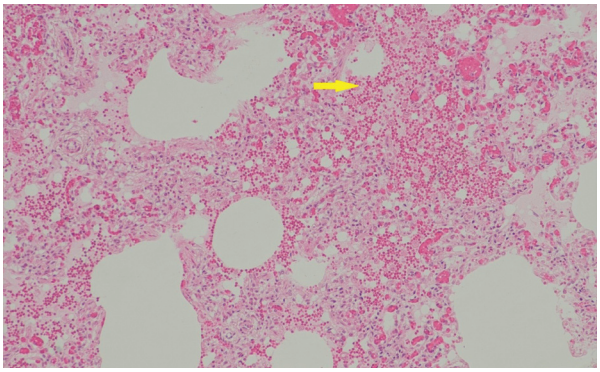

Fig. 2. Haematoxylin and eosin-stained slide of the lungs showing a mixed intra-alveolar infiltrate chiefly composed of macrophages, neutrophils, fresh haemorrhage and oedema.

The second heterozygous single-nucleotide variation, c.5668G>A, is registered on the Atlas of Genetic Cardiac Variation database, with an uncertain clinical significance, likely associated with dilated cardiomyopathy (DCM). The G>A nucleotide change in codon 1890 leads to an amino acid change of glutamic acid (Glu) to lycine (Lys) (p.E1890K) (Fig. 4). This variant is considered by 87.5% of algorithms to be likely damaging, predicting an adverse effect on the protein structure.

The E1890K variant is a non-conservative amino acid substitution, which would likely impact on the secondary protein structure as these residues differ in polarity, charge, size and/or other properties. This substitution occurs at a position that is conserved across species, and *in silico* analysis, predicts this variant to be probably damaging to the protein structure/function. More recently, p.E1890K has been registered on the NCBI database under rs766875593, with an uncertain clinical significance associated with various channelopathies rather than a cardiomyopathy.

Recent studies reported on the identification of this variant in SUID cases associated with the long-QT syndrome (LQTS) as well as Brugada syndrome (BrS). The Genome Aggregation Database (gnomAD) and the Exome Aggregation Consortium (ExAC) reported on an allele frequency of 0.00001 and 0.00002, respectively. However, it is important to note that these allele frequencies are not representative of the African population since no studies have been done to provide statistics on this occurrence.

Wang *et al.*[14] reported on the identification of this variant in a two-month-old infant, whose cause of death could not be determined after a thorough autopsy, scene investigation as well as all ancillary investigations had been conducted. A review of the case history indicated a prone sleeping position at the time of death, with a history of a recent cold, similar to our case study.

Discussion

Two heterozygous missense variations in the *SCN5A* gene were identified in this SUID. Bearing in mind that a cause of death (bacterial pneumonia) had been established for this case prior to genetic testing, the results were deemed significant, although unexpected, in explaining the full circumstances surrounding the death.

The *SCN5A* gene encodes a protein, sodium (Nav1.5) ion channel pore-forming α-subunit, that is expressed only in the myocardium and performs a critical role in heart excitability and conduction.[15-17] The integral membrane protein produces the fast-inward Na+ current that is responsible for the depolarising phase of the cardiac action potential. Variations in this gene cause an increased persistent Na+ current, with a subsequent prolongation of the ventricular action potential, essentially resulting in an inherited predisposition to ventricular arrhythmias and sudden death, seen in several cardiac diseases.[4,18] Previous studies demonstrated a link between SUID and a predominance of *SCN5A* variants, more commonly associated with channelopathies.

Channelopathies are generally described as inherited cardiac arrhythmogenic disorders associated with isolated electric dysfunction caused by variants in genes encoding for cardiac ion channels and regulatory protein receptors, which are involved in the ionic control of the cardiac action potential.[1,14] A link between many human diseases and the dysfunction of ion channels (channelopathies) has been established, either as a result of genetic variants or acquired malfunctions of ion channels.[13,17]

The three most common and epidemiologically relevant genetic heart channelopathies include LQTS, BrS and catecholaminergic polymorphic ventricular tachycardia (CPVT).[1,13,14] Although the involvement of numerous susceptibility genes has been identified, most of the variants (especially in SUID cases) have been located in the *SCN5A* gene, predominantly linked to LQTS and BrS.[13-15] Post mortem genetic studies have implicated channelopathy-associated variants in 10 to 15% of SUID cases.[1,8,14]

The most common clinical manifestations associated with LQTS and BrS are syncope, seizures and sudden death as a result of ventricular arrhythmias, usually occurring during a period of rest/sleep. Of particular note is that these channelopathy-related sudden deaths generally present with no macroscopically identifiable structural alterations of the heart at autopsy.[4,8,15,16] Our case study was found to carry two variations, of which one has been documented with an uncertain clinical significance, although associated with LQTS, BrS and DCM.

Numerous studies have reported on the diversity of the phenotypic and genotypic expression of the *SCN5A* gene with variations linked to other arrhythmogenic disorders, including DCM, progressive familial heart block type 1 and sick sinus syndrome.[8,16,19] Reports of SUID cases where genetic variations associated with cardiomyopathies are increasingly identified in structurally normal hearts should prevent the tendency of eliminating associations between *SCN5A* variations and DCM.[1,8,20-22]

Cardiomyopathies can be described as a group of heart diseases that affect the structure and function of the myocardium, which can all lead to heart failure, arrhythmia and even sudden death.[9,12,23] The most common types of cardiomyopathies include hypertrophic cardiomyopathy, DCM, restrictive cardiomyopathy and arrhythmogenic cardiomyopathy.[12,21,23] Although it is generally associated with cardiac alterations macroscopically identifiable at autopsy, it is not uncommon for a cardiomyopathy to be inadvertently missed in SUID cases, which usually present with a macroscopically normal heart.[1,8,22,23]

Studies have been reported that genetic variations in cardiomyopathy-related genes, which may cause arrhythmia and sudden death, have been identified in SUID cases presenting

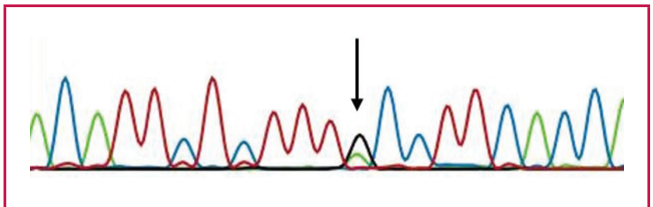

Fig. 3. Heterozygous single-nucleotide variation p.A1856T (c.5566G>A) identified in exon 28 of the *SCN5A* gene.

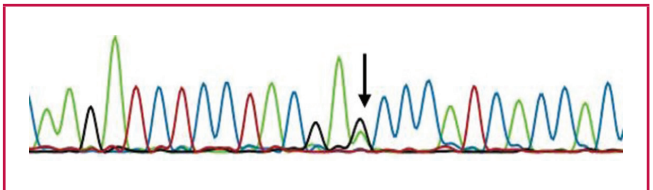

Fig. 4. Heterozygous single-nucleotide variation p.E1890K (c.5668G>A) identified in exon 28 of the *SCN5A* gene.

without any cardiac changes. Research suggest that this might be explained by the progressive nature of cardiomyopathy, whereby in the first stages of the disease the myocardial changes may be so incipient that it may not be visible at autopsy.[1,8,20,24]

The genetic basis of DCM in infants commonly demonstrates phenotypic overlap. Reported cases of DCM due to *SCN5A* variations identified in long-QT syndrome type 3 (LQT3), shows that not only can it result from structural changes in the myocytes, but also from altered calcium ion handling.[10,15,16,19-21] These inherited genetic susceptibilities in infant cases have been proven to play an important role in how the cardiac muscle responds to environmental and infectious factors.[11,22,24]

Researchers believe that variations in the *SCN5A* gene, with its associated higher risk of lethal arrhythmias, are linked to an increase in an infant's critical vulnerability to certain infections. Consequently, acute viral infections are regarded as one of the provocative factors associated with sudden death in infant channelopathy and/or DCM cases.[11,20,23,24] In fact, many of these SUID cases (diagnosed with inherited cardiac arrhythmogenic disorders) demonstrated a viral prodrome within days of their death. Such infants often present with respiratory signs, extreme sleepiness, difficulty in feeding and increased fussiness prior to death.[11,23,24]

It is not uncommon for inherited channelopathies and/or cardiomyopathies in infants to be mistaken for flu, viral upper respiratory tract infection or pneumonia, and without the incorporation of post mortem genetic testing, any other contributory causes of these deaths are often disregarded.[1,8,11,20] Consequently, it is even of greater importance for countries with a high burden of infectious diseases to be especially aware of these findings, as there might be a reasonable tendency to overcall minor findings of viral infection in these SUID cases.[1,2,11,20]

Genetic testing is considered an ideal risk-assessment tool, not only for channelopathies, but for cardiomyopathies as well, due to its ability to identify patients at risk prior to overt disease development.[1,10,22-24] The use of post mortem genetic testing in SUID cases can benefit family members, especially those from poor communities, by providing the first indication of a familial cardiac arrhythmogenic disorder. Ultimately this will allow for the opportunity of preventative intervention, which can be used to avoid the progressive onset of the disease.[8,10,15, 22,23]

For decades now, the undeniable benefit of post mortem genetic testing in SUID cases, especially those that remain unexplained, has been widely recognised worldwide.[1,4,8] The continued advancement in molecular diagnostics and its associated decrease in costs has allowed for expanded molecular testing using cardiac gene panels and next-generation sequencing.[1,4,9,24] Although this is not a novel concept to most first-world countries, it still eludes the radar of many medical professionals practicing in an economically and resource-strained country. These countries, including South Africa, have not yet been conducting post mortem genetic testing in unexplained SUID cases, at least not routinely.[2,3,7,12] The greatest benefit of such testing is not to define the cause of death, but rather the highly disease-specific diagnostic, therapeutic and prognostic benefit derived from subsequent genetic screening of family members of the deceased.[1,8,15,16]

In addition, disease-causing variants in the *SCN5A* gene have been reported as a possible predisposing factor of SUID, providing an apparent aetiology of arrhythmias due to secondary challenges/risk factors such as complicating lower respiratory infections, which are generally tolerated in infants not carrying such genetic variations. Considering South Africa's burden of infectious diseases coupled with a high infant survival rate in most of these cases, a more scrutinised and in-depth investigation into those SUID cases that typically present with no more than minimal findings such as the presence of a mild infection, should be considered.[2,12]

Conclusion

There is a lack of research reporting on the genetics of channelopathies and cardiomyopathies in Africa. The fact that cardiomyopathies are deemed an endemic form of non-communicable diseases, of high importance in the largely low-income communities in SSA, proves the need for local research on this topic. The results from this case study demonstrate the possible impact molecular diagnostics can have on identifying potential inherited cardiac disorders. Additionally, it highlights the occurrence of misdiagnosis of SUID cases in our population, or the possibility of an incomplete understanding pertaining to the circumstances surrounding these deaths. Further molecular testing may provide better knowledge as to why certain infants do not survive these viral and/or bacterial infections.

This case study aimed to create awareness on this subject among medical professionals, especially those practicing in resource-strained countries. Hopefully, this will motivate for more collaborative research and investigation to gain a better understanding of the unique genetic diversity and its associated inherited diseases in SSA.

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