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Forensic Science International: Genetics Supplement Series xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Massively parallel sequencing in sudden unexpected death in infants: A case report in South Africa

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ARTICLE INFO

Keywords:
Molecular autopsy
SIDS
Cardiac arrythmia
SCN10A

ABSTRACT

Sudden unexpected death in infants (SUDI) is a devastating event for a family, and unfortunately occurs relatively frequently in South Africa. These cases are referred for a forensic post-mortem investigation to establish the cause of death; however, despite thorough analyses, some cases remain undetermined. Internationally, a molecular autopsy has assisted in resolving these types of cases by revealing genetic variants which contributed to the demise. Motivated by lack of local research in this field, a study was launched at the University of Cape Town (South Africa) in 2015 to explore the use of molecular autopsies in the medico-legal investigation of local SUDI cases. An ethical framework was established and used to prospectively recruit SUDI cases from one of the busiest forensic facilities: Salt River Mortuary. A next generation sequencing approach was used to assess 43 genes previously associated with cardiac arrhythmias. In a particular infant, a putative pathogenic variant was identified (rs750771811 T/T) in the *SCN10A* gene. The variant is rare, but was homozygous in this infant, and appears to be the first time it has been observed in a SUDI victim. Previous functional studies on the amino-acid residue suggested that this variant may reduce SCN5A activity, which has been linked to Brugada syndrome. A genetic counselling session was arranged with the parents; a full family history was obtained, which revealed that the parents had a previous miscarriage and had recently had a second SUDI. The parents have subsequently been enrolled in the study for genetic screening and have been referred for electrocardiogram assessments. The findings highlight a new possible candidate variant to assess in SUDI cases, and also demonstrate the value of molecular autopsies to families.

1. Introduction

Infant mortality, including sudden unexpected death of infants (SUDI), is particularly high in sub-Saharan Africa [1]. SUDI cases are typically referred for forensic post-mortem investigation, where many for many cases, cause of death is determined [2]. However, some remain unexplained after an autopsy and it has been hypothesised that at least some of these unresolved cases are due to inherited cardiac disorders (e.g. channelopathies and cardiomyopathies) which present with a structurally normal heart [3–5].

As reviewed by various authors, many variants have been linked to inherited cardiac disorders [4,6]. In the forensic context, some of these variants have been found in sudden unexpected death cases, and directly contributed towards the resolution of case [7–9]. It is currently unclear what proportion of SUDI cases in South Africa exhibit underlying inherited cardiac diseases and if unexplained cases could be resolved with the use of a molecular autopsy. As such, a study was established at the University of Cape Town, to investigate the genetic contribution to SUDI in South Africa. A case report is presented here.

2. Methods

A cohort of SUDI cases (n = 201), less than 1 year of age, are continually recruited into the study from Salt River Mortuary (Cape Town, South Africa) with informed consent from next of kin, using an internally-developed ethical framework [10]. Approval was obtained from the Institutional Review Board (HREC: 445/2015). The subset of infants whose cause of death remais undetermined after post-mortem investigation are prioritised for next generation sequencing. In this case example, DNA was prepared for next generation sequencing using the

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https://doi.org/10.1016/j.fsigss.2019.10.051
Received 16 September 2019; Received in revised form 7 October 2019; Accepted 7 October 2019

HaloPlex Target Enrichment System for targeted 43 genes known to be involved in arrhythmia. Preparation was done according to the manufacturer's protocol with no deviations (Agilent Technologies, Santa Clara, USA). The pooled library was thawed and then quantified fluorometically using the GloMax®-Multi detection system (Promega, Madison, USA and then diluted to 6 pM, spiked with 1% 20 pM PhiX (Illumina, Cambridge, UK) and then sequenced using the MiSeq™ Reagent Kit v3 600 cycles PE cartridge (Illumina, Cambridge, UK) on the MiSeq™ System (Illumina, Cambridge, UK).

The FASTQ files were subjected to a series of bioinformatic analyses, which produced a list of variants for each sample. Each variant was assessed and prioritised using Alamut-Visual 2.11.0 and pathogenicity scoring was based on the guidelines of the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) [11]. Prioritised variants were confirmed using Sanger sequencing using the BigDye®Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and capillary electrophoresis using the ABI Genetic Analyser 3130*xl*.

3. Results

A homozygous variant rs750771811 TT in *SCN10A* (NM_006514.2 c.40C > T p.Arg14Cys) was observed in a two month old male of African ancestry. Signs of respiratory tract infection and a structurally normal heart was observed at autopsy, and review of clinical history showed signs of fever prior to death.

The missense variant was considered putatively pathogenic as (i) it was highly conserved across species, (ii) there was a large physiochemical difference between amino acids (iii) it was rare (minor allele frequency: 0.000020), and (iv) *in silico* tools (SIFT, PolyPhen-2 and MutationTaster) predicted pathogenicity.

A genetic counselling session was subsequently arranged with the parents. A full family history was obtained, which revealed that the parents recently had a second SUDI. The parents have subsequently been enrolled in the study for genetic screening and have been referred for electrocardiogram assessments.

4. Discussion

The *SCN10A* gene has been previously linked to BrS [12,13]; however, this association has been widely debated [14–17]. Some studies have demonstrated the functional effect of *SCN10A* on *SCN5A* [13,18,19], with the latter gene having considerably more causal evidence for channelopathies [20,21]. For example, Bezzina et al. (2013) showed that variants in *SCN10A* contributed to BrS by altering the function of the Nav1.5 protein, which is encoded by *SCN5A* [12]. However, Behr et al. (2015) showed that while the minority of rare variants were significantly associated with BrS, a SNP-set (Sequence) Kernel Association Test (SKAT) indicated that overall, the presence of rare variations in *SCN10A* was not significantly different between in cases and controls, particularly in *SCN5A*-negative cases [22]. However, more recently Okamura et al. (2017) showed that variants in *SCN10A* may decrease *SCN5A* expression through DNA methylation in patients without *SCN5A* exonic mutations [19].

Hu et al. (2014) identified 17 variants in *SCN10A* in 25 BrS patients (cohort n = 150) and found that patients with these variants were more symptomatic that those without; and that 16 of the 17 variants were missense. A p.Arg14Leu variant was identified in four unrelated patients, and heterologous co-expression in HEK cells reduced *SCN5A* activity by 79% [23]. In this study, the variant in case 1 (p.Arg14Cys) also affected amino acid number 14 but instead altered the arginine to cytosine.

Considering that the p.Arg14Cys was very rare, it was predicted to be pathogenic *in silico* as well as the functional importance of arginine at residue 14, this variant was considered to *probably* play a role in the infant's demise. This was further supported by the homozygosity of the variant, which has not been reported before, and particularly that this residue functionally affects *SCN5A* activity which is well-linked with channelopathies [24].

The diagnosis of inherited cardiac disorders in sudden unexpected death cases is important for many reasons. From the family's perspective, it provides an explanation as to why their relative passed away, which is essential for closure and acceptance of the death [25]. A molecular diagnosis also allows for a targeted genetic screen in blood relatives who may carry the same mutation(s) and be at risk for sudden death themselves [26,27]. In the case of sudden infant death, this may help parents plan for future pregnancies and better manage the associated risks. In addition to the social and clinical applications, the understanding of contributors towards mortality has value for preventative healthcare, whereby identifying genetic risk factors and associated diseases underlying sudden unexpected death may aid in targeted educational interventions [1,28].

5. Conclusion

This is the first time the c.40C > T variant in *SCN10A* has been observed in a SUDI case. Previous functional studies on the amino acid residue suggest that this variant may reduce SCN5A activity, which has been linked to Brugada syndrome. These findings highlight a new candidate variant to explore using functional studies, as well as to possibly assess in future SUDI cases. This case report also demonstrates the potential value of molecular autopsies to families.

Funding

None

Declaration of Competing Interest

None

Acknowledgments

Salt River Mortuary, Forensic Pathology Service, Western Cape Government, Department of Health.

Susan Louw, Lerato Majara, Kate Reid at the Department of Pathology, University of Cape Town.

Professor Hugh Watkins at the Wellcome Trust Centre for Human Genetics, University of Oxford.

Kate Thomson, Dennis Wan at the Molecular Genetics Laboratories, National Health Services, Oxford University Hospitals.

This research was funded by the RCUK Newton PhD Partnership award, the National Health Laboratory Service Development Trust grant and the Research Office, University of Cape Town.

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