DMPK 3’ untranslated repeat expansions in unexplained sudden cardiac death in the young

Zoe Ward[1,2], Jackson O’Neill[3], Rachael Stiles[4], Andrew Martin[5], Lisa Worgan[6], Jenny Eaton[7], Ian Hayes[7], Laura Yeates[8], Stephanie Burcher[1,7], Jackie Crawford[5], Hui Hui Phua[9], Cris Print[1], Jonathan Skinner[10], Richard D. Bagnall[3,11], Polona Le Quesne Stabej[1]

[1]Molecular Medicine and Pathology, The University of Auckland, New Zealand, [2]Precision Medicine Initiative, The University of Auckland, New Zealand, [3]Bioinformatics and Molecular Genetics at Centenary Institute, The University of Sydney, Australia, [4]Department of Cardiology, Waikato Hospital, Hamilton, New Zealand, [5]Department of Cardiology, Auckland City Hospital, Auckland, New Zealand, [6]Clinical Genetics Service, Institute of Precision Medicine and Bioinformatics, Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia, [7]Genetic Health Service New Zealand-Northern Hub, Te Toka Tumai, Auckland, New Zealand, [8]Genomics and Inherited Disease Program, Garvan Institute of Medical Research and University of New South Wales, Sydney, Australia, [9]Liggins Institute, The University of Auckland, New Zealand, [10]Department of Paediatric and Adolescent Medicine, University of Sydney, NSW, Australia,[11]Faculty of Medicine and Health, The University of Sydney, Australia

Introduction

Sudden cardiac death (SCD) in the young (age 1-35):

- Unexpected death from a cardiac cause
- Frequency across Australia and New Zealand: ~1.3 per 100,000 (1)
- Causes: inherited cardiac diseases, including primary arrhythmogenic disorders and cardiomyopathies
- When no definitive cause of death is found at autopsy, the case is classified as **sudden unexplained death (SUD**), with a presumed arrhythmogenic basis
- Post-mortem cardiac genetic testing uncovers pathogenic variants in only 10–30% of SUD, suggesting other causes may exist. (2,3)

Myotonic dystrophy type 1 (DM1):

- Multi-systemic autosomal dominant disorder caused by the expansion of Short Tandem Repeat (STR): **$[CTG]_n$ repeat** within the **3' UTR (Untranslated Region) of the *DMPK* gene**
- Cardiac arrhythmias are found in 41% of DM1 patients, and SCD occurs in 8% (4)

Detection of STRs in UTRs using short reads technology:

- UTR regions are often not captured in standard cardiac gene panels and exome sequence
- Difficult to accurately determine length as reads do not extend over the expanded repeat
- Require specialised secondary and tertiary analysis

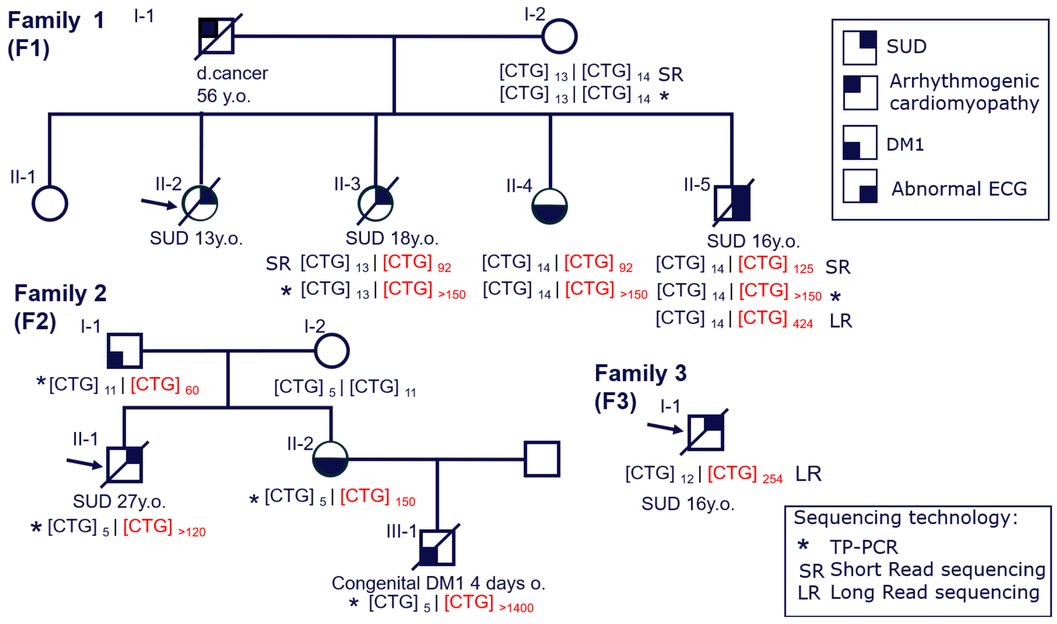
Aim

Identify a genetic cause of sudden death in three families with SUD in the young in which prior genetic testing did not reveal a pathogenic variant.

Results

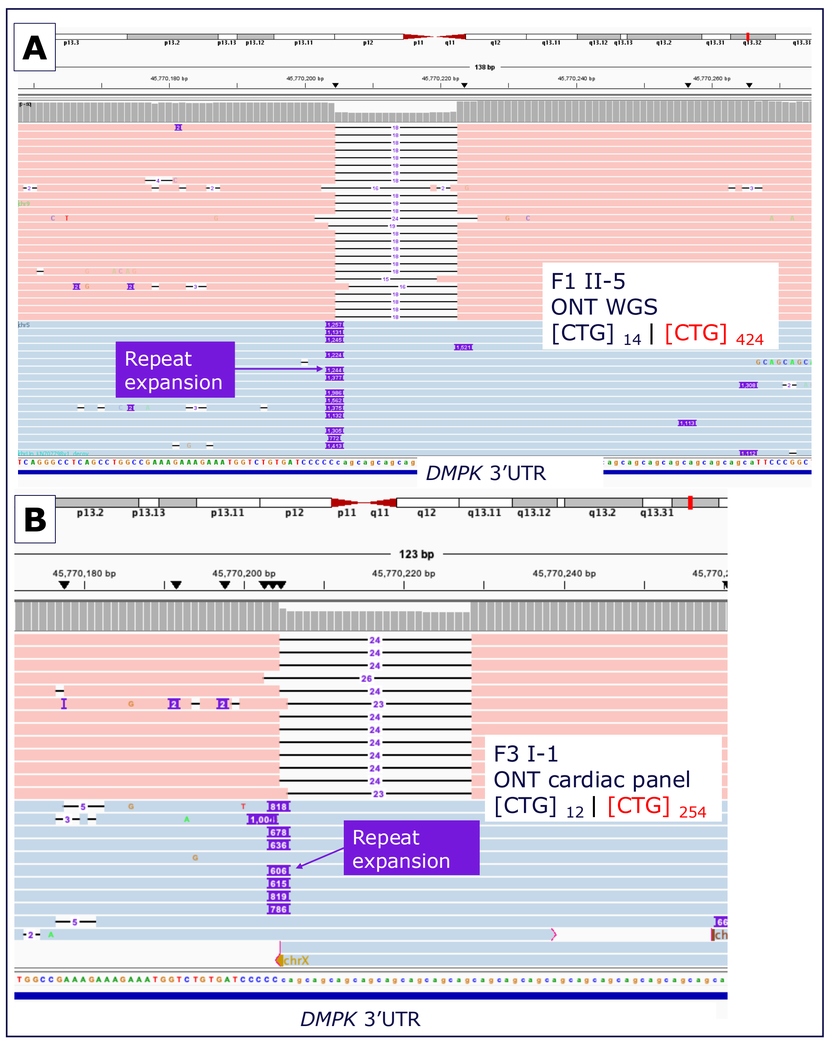
- **Family 1 (F1):** three SUD in young siblings (II-2 and II-5 during exercise, II-3 in verbal altercation); see Fig. 1:
  - Cardiac panels and exome between 1998-2020 did not identify the SUD genetic cause; Whole Genome Sequencing (WGS) with Oxford Nanopore Technology (ONT; **Fig. 2A**) and Illumina (**Fig. 3**)in 2024 identified pathogenic *DMPK* 3’UTR repeat expansion
  - Siblings II-1 and II-4 have preventatively been fitted with an ICD after 3rd SUD (II-3)
  - II-4 has right ventricle (RV) and ECG abnormalities; father (I-1) had arrhythmogenic cardiomyopathy (RV); II-1: RV wall motion abnormality with normal ECG and Exercise Tolerance Test, negative LGE (Late Gadolinium Enhancement), suggesting no evidence for scar/fibrosis
- **Family 2 proband (F2 II-1):** SUD during exercise; see Fig. 1:
  - Exome-based post-mortem cardiac gene panel testing did not identify a genetic cause of disease
  - Proband’s nephew (III-1) died at 4 days old with a clinical suspicion of DM1, confirmed with triplet repeat primed PCR, followed by genotyping in I-1, II-1 and II-2
- **Family 3 proband (F3 I-1):** SUD during exercise; see Fig.1:
  - Suspicion of a *DMPK* repeat expansion from short-read sequencing prompted sequencing with long read ONT cardiac panel and confirmed a pathogenic *DMPK* repeat expansion (**Fig. 2B**)

Fig. 1: Families 1-3 *DMPK* repeat length sizes and phenotypes



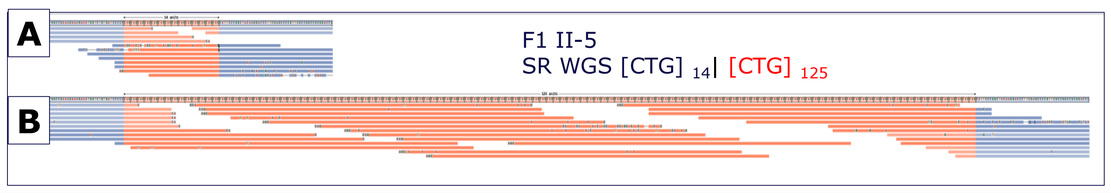
Legend to Fig. 1: Repeats in red: $[CTG]_n$ repeat expansion; repeats in blue: normal $[CTG]_n$ repeat range. Short read sequencing technology: Illumina technology; Long read sequencing: ONT; TP-PCR: triplet-primed PCR. SUD: Sudden unexplained death, y.o.: years old, DM1: Myotonic Dystrophy type 1.

Fig. 2: *DMPK* 3’ $[CTG]_n$ repeat expansion in F1 II-5 (A) and F3 I-1 (B)



Legend to Fig. 2: WGS: Whole Genome Sequence; ONT: Oxford Nanopore Technology. Reads are coloured by haplotypes.

Fig. 3: Visualisation of alignments (WGS, SR/Illumina) of reads using REViwer: *DMPK* $[CTG]_n$ in F1 II-5 depicting normal (A) and expanded (B) allele



Discussion

We report five SUD in young people from 3 families where initial cardiac genetic testing failed to identify a genetic cause, but subsequent investigations revealed pathogenic *DMPK* 3’UTR repeat expansions, years (F2, F3) or decades (F1) later. Our results demonstrate how long-read sequencing spanning the *DMPK* repeat region enables precise repeat sizing (Fig. 2A, 2B); unlike the estimated repeat counts provided by diagnostic tests such as TP-PCR or short-read sequencing (Fig 3). The *DMPK* repeat expansion region is not typically included in cardiac arrhythmia or cardiomyopathy gene panels, and they are challenging to detect with short-read sequencing, requiring secondary analysis with STR genotyping. Our cases highlight the importance of a **comprehensive clinical assessment** of the proband and extended family. Due to the age-related penetrance of DM1, the condition can remain undiagnosed, particularly in children, yet family history may offer critical clues pointing to *DMPK* as a potential cause of SUD.

We recommend that *DMPK 3’* UTR is included in the diagnostic workup for unexplained SUD in the young.

Materials and Methods

Short read whole genome sequencing (WGS):

- F1: I-2, II-2, II-3, II-4, II-5 to 30X coverage using an Illumina platform (Macrogen, S. Korea) and analysed using DRAGEN 4.3 with Expansionhunter for repeat calling

Long-read WGS using Oxford Nanopore Sequencing Technology (ONT):

- F1 II-5 (**Fig.2A**) using PromethION 2 solo at the Liggins Institute (University of Auckland) as described. (5) ONT data was analysed using EPI2ME, using Straglr for STRs (v.1.4.4)

ONT sequencing adaptive sampling 185 cardiac genes (+50kb of flanking regions):

- F3 I-1 (SUD); **Fig. 2B**
- PromethION platform; DNA was extracted using the PacBio Nanobind PanDNA kit

Exome sequencing:

- F2 II-1, F3 I-1

Triplet-repeat Primed (TP) PCR followed by fluorescent-based capillary electrophoresis:

- F1 (I-2, II-2, II-3, II-4, II-5), F2 (I-1, II-1, II-2, III-1), F3 I-1

Acknowledgements

We would like to thank the patients and their families for participating. LabPLUS at Auckland Hospital for TP-PCR. Dr Miriam Rodrigues and A/Prof R. Roxburgh from NZ NeuroGenetic Registry for helpful discussions.
Funding: Pūtahi Manawa (CoRE) and The University of Auckland Foundation. RDB is funded by an NSW Health Senior Scientist Grant.

(1) Bagnall RD, et al. A Prospective Study of Sudden Cardiac Death among Children and Young Adults. New England Journal of Medicine. 2016 Jun 23;374(25):2441–52.
(2) Bagnall RD, et al. Exome analysis-based molecular autopsy in cases of sudden unexplained death in the young. Heart Rhythm. 2014;11(4):655–62.
(3) Martínez-Barrios E, et al. Molecular autopsy: Twenty years of post-mortem diagnosis in sudden cardiac death. Front Med (Lausanne). 2023 Feb 10;10.
(4) Nyaga DM, et al. Benchmarking nanopore sequencing and rapid genomics feasibility: validation at a quaternary hospital in New Zealand. NPJ Genom Med. 2024;9(1):57.
(5) Šabovič M, et al. Relation of CTG expansion and clinical variables to electrocardiogram conduction abnormalities and sudden death in patients with myotonic dystrophy. Neuromuscular Disorders. 2003;13(10):822–6.