RYR2 variant identified in dilated cardiomyopathy and sudden death: results from the 100,000 Genomes Project

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Tweet: *RYR2* variant identified in dilated cardiomyopathy and sudden unexpected death in the young in 100,000 Genomes Project data. #inherited #cardiac #scd

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What is the clinical question being addressed?

Are WGS data from 100,000 Genomes Project effective in diagnosing SUDY victims?

What is the main finding?

A rare *RYR2* variant was found in multiple DCM and SUDY cases. These findings allowed upgrade of the variant to likely pathogenic, opening a potential new disease association.

Sudden unexplained death in the young (SUDY) can be the first presentation of an inherited cardiac condition (ICC) in a family. Therefore, establishing a diagnosis is important for surviving relatives. The 100,000 Genomes Project (100KGP) was launched in 2013 by the United Kingdom government to investigate the diagnostic role of whole genome sequencing (WGS) in a national healthcare system setting. Young sudden death victims with no finding on post-mortem examination and without pre-existing conditions that could explain the event were recruited. All participants provided written informed consent under approval from the HRA Committee East of England - Cambridge South (REC Ref 14/EE/1112). WGS was performed as previously described. Analytical approaches undertaken included: candidate gene approach for protein coding, 5'UTR, and copy number variation in decedents; and trio studies (parents and decedent) with *de novo* analysis of protein coding and intronic variants (Figure 1A). American College of Medical Genetics (ACMG) criteria for variant pathogenicity were applied.(1)

A total of 18 probands were recruited between 2017-2019; three were excluded for not meeting inclusion criteria. The 15 remaining probands had a median age of 18 years [IQR 1-29.5]; two thirds were male. Sixteen family members were recruited, with six full trios. Following all filtering and analysis steps, only one variant was suitable for investigation - *RYR2*:c.10046C>T,p.(Ser3349Leu). This was identified in a 24-year-old female who experienced an out of hospital ventricular fibrillation cardiac arrest with a morphologically

normal heart on post-mortem examination. Family evaluation following her death led to the diagnosis of dilated cardiomyopathy (DCM) in four family members, three were tested for the variant and found to harbor it (Figure 1B(i)).

RYR2:c.10046C>T,p.(Ser3349Leu) variant has an ACMG classification of unknown significance (VUS): PM2-moderate, absent from controls; PP3-supporting, multiple pathogenic computational predictions. Review of other (non-SUDY) 100KGP participants identified this variant in an additional participant recruited for the DCM cohort. The participant's son died during a heart failure admission and was found to have DCM on post-mortem examination and harbored the same missense *RYR2* variant (Figure 1B(ii)). An additional family with SUDY and DCM phenotype was identified with this variant from our ICC clinic (Figure 1B(iii)).

Two of the three deaths in the three families occurred suddenly and at rest. Imaging was available for one proband and for four family members hosting the variant. The proband had severe biventricular systolic dysfunction (LVEF and RVEF = 10%) and left ventricular end diastolic volume (EDV) of 410 ml and right ventricular EDV of 344 ml. Family members demonstrated a mean left ventricular ejection fraction (LVEF) of 45.5±4.2% and left ventricular diastolic diameter of 49.3±4.2mm. One family member had a past medical history of aortic valve replacement, experienced ventricular arrhythmias, and received a cardiac resynchronization therapy ICD.

RYR2 pathogenic variants are usually associated with normal cardiac structure and the risk for sudden death, as is the case in catecholaminergic polymorphic ventricular tachycardia (CPVT). (1) And yet we identified a *RYR2* missense variant in multiple subjects with DCM phenotype and/or SUDY. An extensive literature search then identified the same *RYR2* variant in DCM cases and in SUDY victims, albeit in conjunction with other putative variants in some.(2-5) Based on these findings, the variant can be re-classified as likely pathogenic by

adding PP1-moderate and PP4-supporting criteria, resulting in the discovery of a potential new disease association. Functional studies are now necessary to better characterize the variant's effect.

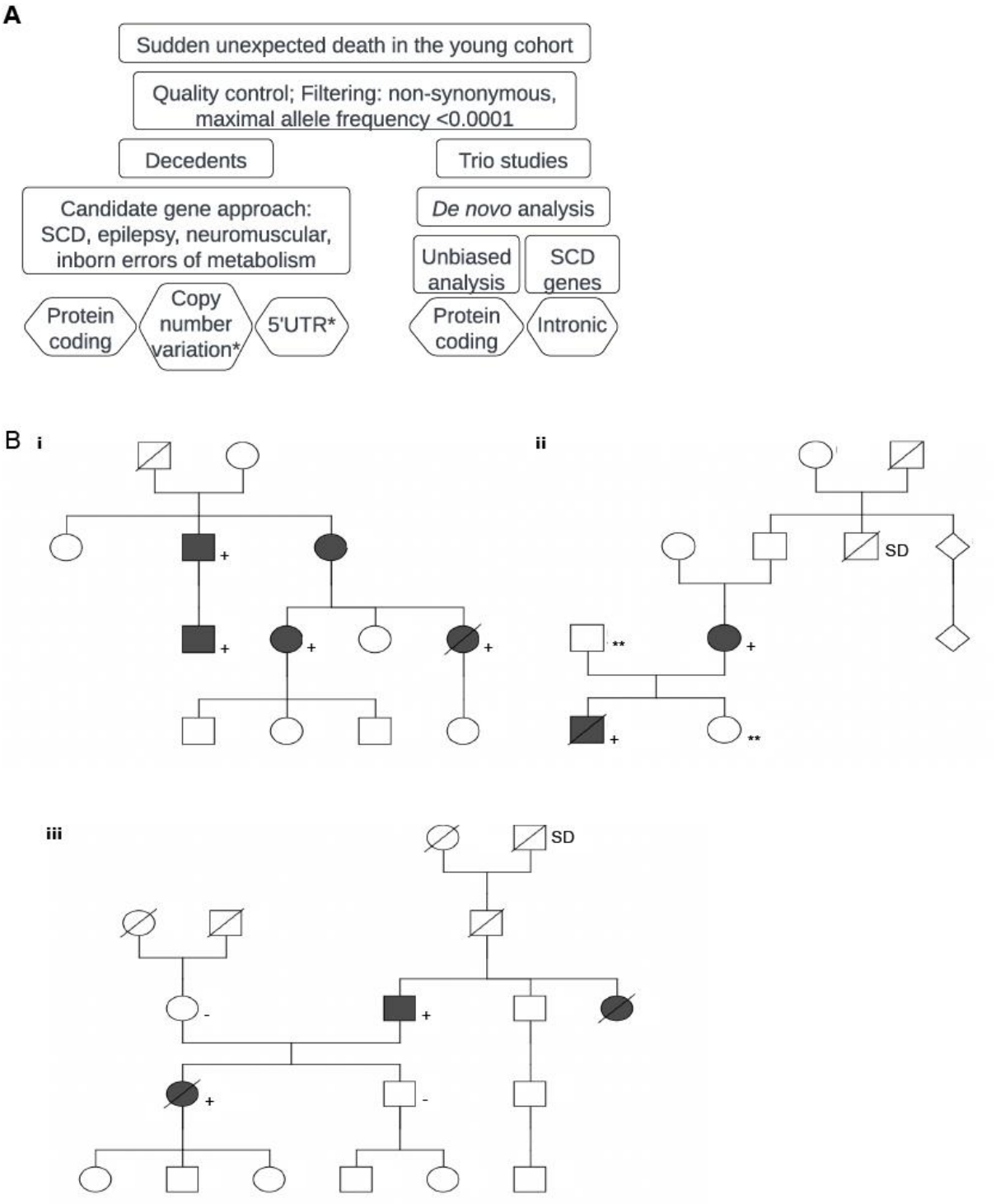

Figure 1A. Analysis methods. Following quality control and filtering decedents data were analyzed using a candidate gene approach for protein coding regions, 5'UTR and copy number variations (*SCD genes only). *De novo* analysis was performed for trio studies. SCD, sudden cardiac death.

B. Families harboring *RYR2*:c.10046C>T,p.(Ser3349Leu) variant. (i)Initial family identified through SUDY cohort in 100KGP. **(ii)**Family identified through the DCM cohort of 100KGP. The proband's partner and daughter (**) show a mild DCM phenotype and have not had genetic testing. (iii)Additional family identified in our ICC clinic. +indicates harboring *RYR2*:c.10046C>T,p.(Ser3349Leu), -indicates negative test; black-filled symbols indicate DCM phenotype. SD, sudden death.

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