Yield of Molecular Autopsy in Sudden Cardiac Death in Athletes. Data from a Large Registry in the United Kingdom

Gherardo Finocchiaro[a] MD, PhD*, Davide Radaelli[a,b] MD*, David Johnson[a] PhD, Raghav T. Bhatia[a] MBBS, MRCP, Joseph Westaby[a] MBBS, Stefano D'Errico[b], MD, PhD, Michael Papadakis[a] MBBS, FRCP, MD, Sanjay Sharma[a] BSc, MBChB, FRCP, MD, Mary N. Sheppard[a] MBBCH, BAO, BSc, MD, FRCPath, Elijah R. Behr[a] MA, MBBS, MD, FRCP

Institutions:

[a] Cardiovascular Clinical Academic Group and Cardiology Research Section, St. George's, University of London, St. George's University Hospitals NHS Foundation Trust, United Kingdom

[b] Department of Medicine, Surgery and Health, University of Trieste, Trieste, Italy

Word count: 853 words

* equally contributed as first author

Author for correspondence:

Professor Elijah R Behr,
Professor of Cardiology, St. George's University of London, Cardiovascular Sciences,
Cranmer Terrace, London, SW17 0RE, UK.
E-mail: ebehr@sgul.ac.uk

Conflict of interest: The authors have nothing to disclose

Abstract

Background: Sudden cardiac death (SCD) may occur in apparently healthy individuals, including athletes. We report the diagnostic role of post-mortem genetic testing, molecular autopsy (MA), in elucidating the cause of SCD in athletes.

Methods: We reviewed a database of 6860 consecutive cases of SCD referred to our specialist cardiac pathology centre. All cases underwent detailed cardiac autopsy and 748 were deemed to be athletes. Of these, 42 (6%) were investigated with MA (28 using a targeted sequencing, 14 exome sequencing). Variants were classified manually as pathogenic (P), likely pathogenic (LP), variant of unknown significance (VUS) using international guidelines. Clinical information was obtained from referring coroners who completed a detailed health questionnaire.

Results: Out of the 42 decedents (average age 35 years old, 98% males) who were investigated with MA, the autopsy was in keeping with a structurally normal heart (sudden arrhythmic death syndrome, SADS) in n=33 (78%) cases, followed by arrhythmogenic cardiomyopathy (ACM) in 8 (19%) individuals and idiopathic left ventricular fibrosis in 1 (2%). Death occurred during exercise and at rest in 26 (62%) and 16 (38%) individuals respectively. Variants that were adjudicated clinically actionable were present in 7 cases (17%). There was concordance between the genetic and phenotypic findings in 2 cases of ACM. None of the variants identified in SADS cases were previously linked to channelopathies. Clinically actionable variants in cardiomyopathy-associated genes were found in 5 cases of SADS.

Conclusions: The yield of MA in athletes who died suddenly is 17%. In SADS cases, clinically actionable variants were found in cardiomyopathy-associated genes and not in

channelopathy-associated genes. ACM is a common cause of SCD in athletes and one in four decedents had a clinically actionable variant in FLNC and TMEM43 genes.

Keywords: Molecular autopsy, sudden cardiac death.

Sudden cardiac death (SCD) may occur in apparently healthy individuals including athletes. The yield of post-mortem genetic testing (molecular autopsy - MA) in athletes who died suddenly is unknown[1]. We aimed to assess the yield of pathogenic (P) and likely pathogenic (LP) variant by MA in a cohort of athletes who died suddenly and underwent post-mortem examination by a expert cardiac pathologists.

We reviewed a database of 6860 consecutive cases of SCD referred to our specialist cardiac pathology centre at St George's, University of London between 1994 and 2020. SCD was defined as death from a cardiovascular cause within one hour of onset of symptoms if witnessed, or within 12 hours of onset if unwitnessed. Clinical information was obtained from referring coroners who were asked to complete a detailed health questionnaire. We arbitrarily defined athletes as individuals that engaged in at least 5 hours of organised exercise activity per week. All cases underwent detailed post-mortem evaluation of the heart, including histological analysis, by expert cardiac pathologists (MNS, JW). A minimum of 10 blocks of tissue were taken for histological analysis and cardiomyopathy was defined as reported previously[1,2]. Death during exercise was defined as occurring while the individual was engaging in exercise, as opposed to death during daily activities or rest. MA was performed with targeted panel and whole exome sequencing focusing on a broad panel of genes implicated in channelopathy and cardiomyopathy as previously described[3]. Variants were classified manually as P, LP or a variant of unknown significance (VUS) using the American College of Medical Genetics and Genomics (ACMG) consensus statement

guidelines[4]. Ethical and research governance approval have been granted for this study (10/H0724/38). Results are expressed as mean ± standard deviation (SD) for continuous variables or as number of cases and percentage for categorical variables.

Out of the total cohort, 748 individuals were athletes. A minority of consecutive athletes with DNA available (n=42, 6%) were investigated with MA: 28 individuals with targeted panel sequencing and 14 with whole exome sequencing. The average age was 35 ± 11 years old and 98% (n=41) were male. A structurally normal heart at the post-mortem examination with negative toxicology (sudden arrhythmic death syndrome - SADS) was found in 33 (78%) decedents. Arrhythmogenic cardiomyopathy (ACM) was observed in 8 (19%) cases and idiopathic left ventricular fibrosis in 1 (2%). Death occurred during exercise in 26 (62%) cases and at rest in 16 (38%), including 5 (12%) cases where death occurred during sleep. MA showed P/LP variants in 9 (21%) individuals. Variants that were adjudicated as the likely cause of death were present in 7 cases (17%). Two cases had a P/LP variant in *HFE* in heterozygous state unlikely to cause haemochromatosis. There was concordance between the genetic and phenotypic findings in two cases of ACM (Table 1). No P/LP variants linked to channelopathies were identified in the SADS cases (Table 1).

Athletes often appear to epitomize health but SCD may occur in apparently healthy individuals[1,5]. Post-mortem examination is a crucial diagnostic step in establishing the cause of SCD and in guiding the clinical evaluation of surviving relatives[6,7,8,9]. The interpretation of the post-mortem results, however, can be challenging especially when the heart is structurally normal or when abnormalities of uncertain significance are found[10]. MA has the potential to establish the cause of death[11,12]. Indeed, a study from our group on a large cohort of SADS decedents investigated with MA found a clinically actionable pathogenic or likely pathogenic

variants in 13% of the cases, mostly associated with channelopathy[3]. Our study comprised 42 athletes where the post-mortem examination performed by expert cardiac pathologists was mostly in keeping with a structurally normal heart and ACM. Roughly one fifth of athletes had a pathogenic or likely pathogenic variant. Clinically actionable variants were found in 17% of cases. Interestingly, none of the athletes with SADS were found to have P/LP variants associated with channelopathies. In all cases P/LP variants were identified in cardiomyopathy-associated genes. This suggest that cardiomyopathy, even when 'concealed' and not detected at expert cardiac autopsy, may predispose to SCD in young male athletes. This is in line with a recent study on 91 autopsy-inconclusive SCD cases where cardiomyopathy-associated genes harboured 70% of clinically actionable variants[13]. Genetic findings correlated with the phenotype in only two cases, both with ACM; *FLNC* and *TMEM43* were the genes involved, which emphasizes their arrhythmic risk of these specific disorders[14,15]. Although our cohort is large, not all cases of SCD are referred to our centre and this introduces a bias. Further, only 6% of athletes who died suddenly and were referred to our centre, were investigated with MA. This implies potential selection bias which should be taken in account when interpreting the results.

In conclusion, in a small cohort of athletes who died suddenly and who were investigate with post-mortem genetic test, the yield of MA was 17%. In SADS cases P/LP variants were found in cardiomyopathy-associated genes and not in channelopathy-associated genes. Genetic panels should include assessment of genes implicated in cardiomyopathy even when a clear phenotype is not identified through post-mortem examination. One in four decedents with arrhythmogenic cardiomyopathy were identified with P/LP variants.

Acknowledgements: We thank the charitable organisation, Cardiac Risk in the Young (CRY)

Funding sources: RTB and GF are funded by CRY. Drs Behr received research funding from the Robert Lancaster Memorial Fund, British Heart Foundation (BHF) and have been sponsored by McColl's Retail Group Ltd, UK

Conflict of interest: none declared.

Data Availability Statement: Data supporting this study are available from CRY Centre for Cardiac Pathology in St. George's University, London. Access to the data is subject to approval and a data sharing agreement due to ethical reason.

References

1. Finocchiaro G, Papadakis M, Robertus J-L, Dhutia H, Steriotis AK, Tome M, et al. Etiology of Sudden Death in Sports: Insights From a United Kingdom Regional Registry. *J Am Coll Cardiol.* 2016;67(18):2108-2115. doi:10.1016/j.jacc.2016.02.062
2. Basso C, Aguilera B, Banner J, Cohle S, d'Amati G, de Gouveia RH, et al. Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology. *Virchows Arch.* 2017;471(6):691-705. doi:10.1007/s00428-017-2221-0
3. Lahrouchi N, Raju H, Lodder EM, Papatheodorou E, Ware JS, Papadakis M, et al. Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome. *J Am Coll Cardiol.* 2017;69(17):2134-2145. doi:10.1016/j.jacc.2017.02.046
4. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424. doi:10.1038/gim.2015.30
5. Corrado D, Basso C, Rizzoli G, Schiavon M, Thiene G. Does sports activity enhance the risk of sudden death in adolescents and young adults? *J Am Coll Cardiol.* 2003;42(11):1959-1963. http://www.ncbi.nlm.nih.gov/pubmed/14662259.
6. Osborn M, Lowe J, Sheppard MN SS. Guidelines on autopsy practice: sudden death with likely cardiac pathology. *R Coll Pathol.* 2015;G145.

7. Finocchiaro G, Dhutia H, Gray B, Ensam B, Papatheodorou S, Miles C, et al. Diagnostic yield of hypertrophic cardiomyopathy in first-degree relatives of decedents with idiopathic left ventricular hypertrophy. *Europace*. February 2020. doi:10.1093/europace/euaa012
8. Wilde AAM, Semsarian C, Márquez MF, Sepehri Shamloo A, Ackerman MJ, Ashley EA, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the state of genetic testing for cardiac diseases [published correction appears in Europace. 2022 Aug 30;:]. *Europace*. 2022;24(8):1307-1367. doi:10.1093/europace/euac030
9. Behr ER, Scrocco C, Wilde AAM, Marijon E, Crotti L, Iliodromitis KE, et al. Investigation on Sudden Unexpected Death in the Young (SUDY) in Europe: results of the European Heart Rhythm Association Survey. *Europace*. 2022;24(2):331-339. doi:10.1093/europace/euab176
10. Papadakis M, Papatheodorou E, Mellor G, Raju H, Bastiaenen R, Wijeyeratne Y, et al. The Diagnostic Yield of Brugada Syndrome After Sudden Death With Normal Autopsy. *J Am Coll Cardiol*. 2018;71(11). doi:10.1016/j.jacc.2018.01.031
11. Crotti L, Brugada P, Calkins H, Chevalier P, Conte G, Finocchiaro G, et al. From gene-discovery to gene-tailored clinical management: 25 years of research in channelopathies and cardiomyopathies. *Europace*. 2023;25(8):euad180. doi:10.1093/europace/euad180
12. Ben-Haim Y, Behr ER. Genetics of sudden cardiac death. *Curr Opin Cardiol*. 2022;37(3):212-218. doi:10.1097/HCO.0000000000000946
13. Isbister JC, Nowak N, Yeates L, Singer ES, Sy RW, Ingles J, et al. Concealed Cardiomyopathy in Autopsy-Inconclusive Cases of Sudden Cardiac Death and Implications for Families. *J Am Coll Cardiol*. 2022;80(22):2057-2068. doi:10.1016/j.jacc.2022.09.029
14. Gigli M, Stolfo D, Graw SL, Merlo M, Gregorio C, Nee Chen S, et al. Phenotypic Expression, Natural History, and Risk Stratification of Cardiomyopathy Caused by Filamin C Truncating Variants. *Circulation*. 2021;144(20):1600-1611. doi:10.1161/CIRCULATIONAHA.121.053521
15. Dominguez F, Zorio E, Jimenez-Jaimez J, Salguero-Bodes R, Zwart R, Gonzalez-Lopez E, et al. Clinical characteristics and determinants of the phenotype in TMEM43 arrhythmogenic right ventricular cardiomyopathy type 5. *Hear Rhythm*. 2020;17(6):945-954. doi:10.1016/j.hrthm.2020.01.035

Table 1. Genetic test results in athletes who died suddenly. All variants were heterozygous. The first seven cases (light grey shading) are deemed to harbour an actionable variant.

Case	Gene	Variant details	p. annotations	ACMG Interpretation	ClinGen	Genecards	ClinVar	PM diagnosis	Concordance
1	FLNC	c.4718T>A	p.Leu1573Ter	Pathogenic	HCM – Definitive (AD) DCM- Definitive (AD) Myofibrillar Myopathy		HCM-definitive (AD) DCM-definitive (AD) Myofibrillar Myopathy	Arrhythmogenic cardiomyopathy involving both ventricles	YES
2	TMEM43	c.1073C>T	p.Ser358Leu	Pathogenic	AC – Definitive (AD)		AC	Arrhythmogenic Cardiomyopathy	YES
3	CDH2	c.2060A>G	p.Asn687Ser	Likely Pathogenic	AC – Limited (AD)	Agenesis of corpus callosum, cardiac, ocular, and genital syndrome	Not present specific variant	SADS	NO
4	CDH2	c.586A>G	p.Ser196Gly	Likely Pathogenic	AC – Limited (AD)	Agenesis of corpus callosum, cardiac, ocular, and	Not present specific variant	SADS	Disputed – Slight fibrosis of RV interpreted as not significant.

						genital syndrome			
5	TTN	c.9577C>T	p.Arg3193Ter	Pathogenic	DCM – Definitive (AD) TTN-related myopathy – Definitive (AR) ACM – Limited (AD) HCM – Limited (AD)		DCM - Conflicting interpretations of pathogenicity: Likely pathogenic; Uncertain significance	SADS	NO – No evidence of cardiomyopathy
6	CRYAB	c.32G>A	p.Arg11His	Likely Pathogenic	/	Myofibrillar myopathy, DCM	DCM – Conflicting - VUS 2 submitters – LP 1 submitter	SADS	NO –No evidence of cardiomyopathy
7	SOS1	c.3392G>A	p.Arg1131Lys	Pathogenic	Noonan Syndrome– Definitive (AD) Costello Syndrome– Disputed (AD) Cardiofaciocutaneus Syndrome - Disputed (AD)	Noonan Syndrome, Gingival fibromatosis	RASopathy (Uncertain significance) Noonan syndrome (Uncertain significance)	SADS	NO –No evidence of cardiomyopathy

8	HFE	c.845G>A	p.Cys282Tyr	Pathogenic	Type 1 hemochromatosis (Gene Associated with Autosomal Recessive Phenotype)	Type 1 hemochromatosis, Microvascular complications of diabetes	Cardiomyopathy (NOS)	SADS	NO
9	HFE	c.845G>A	p.Cys282Tyr	Pathogenic	Type 1 hemochromatosis (Gene Associated with Autosomal Recessive Phenotype)	Hemochromatosis, Type 1 Microvascular Complications of Diabetes	Cardiomyopathy (NOS)	Arrhythmogenic cardiomyopathy (predominant RV involvement)	NO

Abbreviations: ACM: arrhythmogenic cardiomyopathy, AD: autosomal dominant, AR, autosomal recessive, DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy; PM: post-mortem; RV: right ventricle, SADS: sudden arrhythmic death syndrome, VUS: variant of uncertain significance; NOS: not otherwise specified; LP: likely pathogenic; RV: right ventricle; ACMG: American College of Medical Genetics; ClinGen: Clinical Genome Resource https://clinicalgenome.org/; ClinVar: NCBI database https://www.ncbi.nlm.nih.gov/clinvar/. The "Concordance" column indicates whether there is concordance between the genetic data and the morphological findings.

Figure 1. Molecular autopsy in sudden cardiac death in athletes. Genes involved are described. Seven actionable variants have been found in 7 athletes.

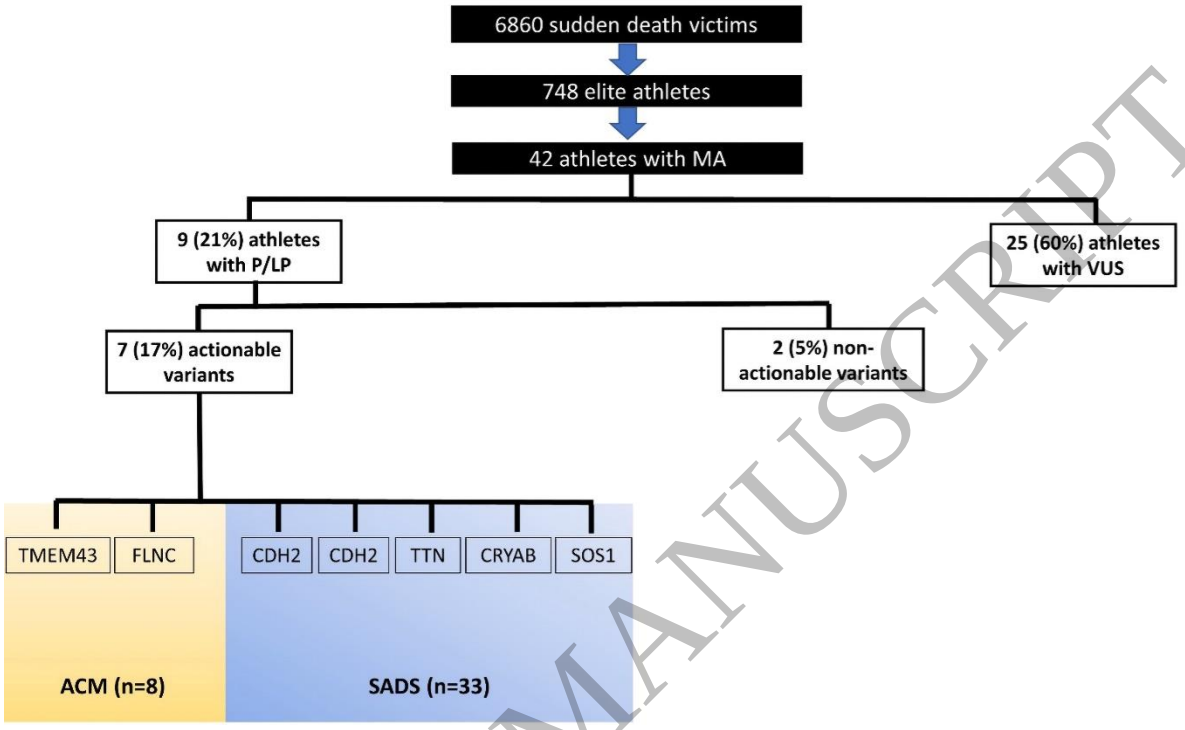

Figure 1
201x121 mm (x DPI)

Abbreviations: MA: Molecular Autopsy; ACM: arrhythmogenic cardiomyopathy; LP: likely pathogenic variant; P: pathogenic variant; VUS: variant of uncertain significance; SADS: sudden arrhythmic death syndrome.