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# **Surviving Sudden Death: Where Does Next Generation Sequencing Fit in the Assessment of Sudden Death Victims and their Families**

**Running title:** *Hamilton et al.; Post-mortem Sequencing for Sudden Death*

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The investigation of sudden death is one of the few enduring responsibilities of the Coronial system that had its origins in 11<sup>th</sup> century Britain and was formally established by the articles of Eyre in 1194.<sup>1</sup> Persons finding a body from a sudden or unnatural death were required to raise the "hue and cry" and to notify the coroner.

Although the familial nature of sudden death, including from structural and electrical cardiomyopathies, has been recognized for many decades or more, British pathologist MJ Davies<sup>2</sup> in 1999 may be the first to suggest that the family might be approached in the evaluation of sudden cardiac death. In the same year, Ackerman and colleagues<sup>3</sup> used molecular diagnosis to identify the cause of sudden cardiac death in a 19-year-old who died after near-drowning, heralding the era of what would be called the molecular autopsy. (Of note, the decedent's mother had a definitely prolonged QT interval) Shortly thereafter, clinical genetic testing for inherited arrhythmia conditions became increasingly available.

Potential approaches to identifying heritable causes of sudden cardiac death include family assessment, molecular assessment or a combined approach. (See table)<sup>4-16</sup> Behr and colleagues<sup>4</sup> clinically evaluated 147 first-degree relatives of 32 sudden arrhythmia death syndrome (SADS) victims with a 22% diagnostic yield for the cause of SCD, whereas more recently, in a larger cohort of victims, a 13.5% yield was identified.<sup>5</sup> Recent studies of the molecular autopsy approach using gene panels of varying sizes<sup>6-8</sup> or whole exome sequencing<sup>9</sup> have identified varying diagnostic yields averaging of 13%. Large studies using a combined approach of family assessment combined with molecular diagnosis of decedent and/or family members provided larger diagnostic yields than family or molecular assessment alone, with an averaged combined diagnostic yield of 31%.

In the current study,<sup>17</sup> Lin and colleagues have performed an evaluation of 89 cardiac

channelopathy and cardiomyopathy genes in a sudden unexpected death cohort of 296 decedents, applying a statistical framework to filter candidate causal variants based on factors that include prevalence and penetrance of the diseases related to those variants<sup>18</sup> and reporting the results according to the recent ACMG framework.<sup>19</sup> Using these stringent guidelines, they identified 17 pathogenic or likely pathogenic (P/LP) variants in 16 subjects, or 5.4% of their cohort. However, the authors also identify 46 *novel* variants, and 130 variants with allele counts lower than that expected on the basis of their related disease. This finding demonstrates the high stringency of ACMG guidelines, for which novelty or rarity represent only a single moderate criterion for pathogenicity. In the absence of family data (identifying a de novo or segregating status for the variant) or a well-established functional assay, such variants will not fulfill P/LP status.

A specific comparison to the recent study of Lahrouchie and colleagues<sup>11</sup> is warranted, as that study also applied ACMG criteria. Lin et al.<sup>17</sup> used GNOMAD instead of EXAC and this may have given rise to different minor allele frequencies that may have altered yield in the Lahrouchi paper. There is lack of data on frequent rare variants or disease associated variants in non-Caucasian ethnic groups. The Lahrouchi study was predominantly white Caucasian compared to 50% African American in the current study. The lack of available family data limited the ability to upgrade VUSs to P/LP. This was helpful with supporting pathogenicity for a number of novel variants using family segregation or confirmation of de novo variants in the Lahrouchie paper.

In parallel with sudden cardiac death investigation, a system for investigation of survivors of sudden cardiac arrest (SCA) and their family members is becoming increasingly important. Assessment of SCA survivors (and their family members) may provide an even higher

diagnostic yield than SCD victims,<sup>14</sup> as the proband demonstrating the clearest disease penetrance is thus available for both detailed clinical and genetic assessment. In the assessment of such cardiac arrest survivors, clinical assessment again appears to provide a higher diagnostic yield<sup>15</sup> than genetic assessment alone.<sup>16</sup>

The authors are somewhat unique as a large medical examiner's office performing their own sequencing and variant interpretation, as opposed to most coroner/M.E. systems that use commercial labs for this work. While part of the rationale for this is the wide variation of reporting from commercial laboratories, representatives from such labs did contribute to ACMG guidelines and most are now using the ACMG framework for reporting. It seems infeasible for small to moderate Coroner/ME programs to reproduce the described system. It would be of interest to know how the variant identification and interpretation process reported compares to that within heritable heart disease clinics in the New York City region, and whether hospital based clinics have to repeat or 'reinterpret' this process once a patient is sent for consultation. (A potential problem if systems are not integrated)

While coroners and medical examiners should provide an opinion regarding what cardiac disease was or might have been present following a detailed examination and death investigation, this should be seen as only the beginning of the assessment. The evaluation of the family (in which genetic contribution are suspected to have played a role) in subspecialty clinics provides another layer of information that is complementary to death investigation, and aligns the responsibility of identifying a familial cardiac condition with those who will care for that family going forward.

Beyond sequencing and variant interpretation, coroner and ME offices and pathologists aim to improving recognition of appropriate pathological entities by integrating investigations

with pathological examinations (getting the phenotype right), keeping the needs of inherited arrhythmia clinics in mind and maximizing information transfer, communicating with families and encouraging families to attend those clinics.

It is equally critical that geneticists and cardiologists embrace the efforts of death investigation systems to assist with these cases. A fruitful approach for the clinical community may involve guiding the efforts of death investigators through education, highlighting examples of appropriately integrated systems and actively reaching out to pathologists and coroners to improve collaboration and integration of their activities into clinical practice guidelines to establish a 'standard of care'. The cardiac pathology community is a great bridge in this endeavour.

It may be neither appropriate nor rewarding to wait for a molecular autopsy result. After an appropriate mourning period, family evaluation as advised by guidelines<sup>20</sup> yields important clinical diagnoses.<sup>4,10,12</sup> The overall yield of clinical diagnoses in SADS families is approximately 30% when summarizing currently known studies.<sup>21</sup> Lahoruchi et al<sup>11</sup> found that in 82 families diagnoses were made in 29% with clinical evaluation and 22% with molecular autopsy. Combined this yielded 39% of families with clinical and/or molecular diagnoses, with 8-9% sharing clinical and molecular diagnoses. Ideal management therefore requires both molecular autopsy and family evaluation to achieve the optimal findings.

**Disclosures:** none

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**Table:**

<b>Family Assessment Only</b>									
<b>Year</b>	<b>Author</b>	<b>Jurisdiction</b>	<b>Date Range</b>	<b>Subjects</b>	<b>Number</b>			<b>Clinical Yield</b>	
2003	Behr et al. <sup>4</sup>	England	2002	sudden arrhythmic death syndrome (SADS)	32 (147 1° relatives)			22% (7/32)	
2014	Giudici <sup>5</sup>	London and Milan	2003-2013	autopsy-negative SUD (1 to 50 yr.)	52 families			13.5% (7/52)	
<b>Genetic Assessment Only</b>									
<b>Year</b>	<b>Author</b>	<b>Jurisdiction</b>	<b>Date Range</b>	<b>Subjects</b>	<b>Number</b>	<b>Genes</b>	<b>Genetic Yield</b>		
2017	Dewar et al. <sup>8</sup>	Manitoba	1998-2013	autopsy-negative child SUD ≤5 yrs.	191	71	6.3%		
2014	Wang et al. <sup>6</sup>	New York City	2008-2012	autopsy-negative SUD (0 to 58 yr.)	274 (141<1yr.)	6	13.5% to 19.8%		
2014	Bagnall et al. <sup>9</sup>	Sydney	2005-2009	SUD age 1 to 40 yr.	28	Exome	10% to 31%		
2015	Farrugia et al. <sup>7</sup>	Strasbourg		autopsy-negative SUD age <35 yr.	16	22	18.8%		
<b>Genetic and Family Assessment</b>									
<b>Year</b>	<b>Author</b>	<b>Jurisdiction</b>	<b>Date Range</b>	<b>Subjects</b>	<b>Number</b>	<b>Genes</b>	<b>Genetic Yield</b>	<b>Clinical Yield</b>	<b>Combined Yield</b>
2005	Tan et al. <sup>18</sup>	Amsterdam	1996-2003	sudden unexpected death age < 40 yrs.	43 families	targeted based on phenotype	23% (10/43)	40% (17/43)	40% (17/43)
2017	Lahrouchi et al. <sup>13</sup>	Multiple	2000-2015 Overlapping Cohorts	autopsy-negative SUD age 1 to 68 yrs.	302	77	13% (40/302) 22% 18/82	26% (21/82)	39% (32/82)
2008	Behr et al. <sup>19</sup>	St. George's Hospital, London		autopsy-negative SUD age 4 to 64 yrs.	57 families	12*	14% (8/57)	51% (29/57)	53% (30/57)
2013	Hofman et al. <sup>13</sup>	Amsterdam	1996-2011	sudden unexpected death age < 45 yrs.	372 families	targeted based on phenotype	18% (67/372)	25% (93/372)	29% (108/372)
2013	Kumar et al. <sup>14</sup>	Melbourne	2007-2012	SUD	109	targeted based on phenotype			18% (19/109)
<b>Cardiac Arrest Survivors</b>									
2013	Kumar et al. <sup>14</sup>	Melbourne	2007-2012	SCA Survivor	52	targeted based on phenotype			62% (32/52)
2016	Herman et al. <sup>15</sup>	Canada	2004-2013	SCA Survivor age 18 to 88 yrs.	200			34 to 41%	
2017	Mellor et al. <sup>16</sup>	Canada	2006-2015	SCA Survivor	174	targeted based on phenotype	17% (29/174)		