The molecular autopsy: Identification, verification and reporting of genetic markers associated with sudden infant death syndrome and young sudden unexplained death victims.

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Abstract

Each year in the United States thousands of cases of sudden and unexpected deaths of infants, children, and young adults are assigned an undetermined cause of death after postmortem investigation and autopsy. Heritable genetic variants have been suggested as the cause of up to a third of sudden death (SD) cases. In order to develop cost-effective genetic screening for autopsy, we sequenced full exons of 64 genes associated with SD in the largest known cohort (351) of infant and young SD decedents using next-generation sequencing technology at less than $600 per sample. Putative causal genetic variants were assessed through literature review and clinical evaluation by a multidisciplinary consortium of experts. Thirteen individuals (3.7%), 8 infants (2.8% of those < 1 year of age) and 5 children/young adults (7.0% of those >1 year of age), were found to have a causal genetic variant resulting in SD. These variants were observed in 10 of 64 genes tested. Combined with the large cohort size and multidisciplinary screening of putative pathogenic genetic variants, these percentages represent an estimate lower than those previously reported. This study demonstrates a strategy to implement thorough molecular autopsies in medicolegal investigations of young SD decedents.

Table of Contents

Executive Summary **4**

Purpose **4**

Research Design **5**

Findings **7**

Conclusions **8**

I. Introduction **12**

II. Methods **16**

Cohort 16

DNA extraction 16

Capture array design and validation 17

Sample entry, library preparation, target capture, and sequencing 17

Primary data analysis 19

Variant filtration 19

Clinical genetic variant confirmation 22

III. Results **23**

Cohort demographics 23

Genetic variants 24

Data access 26

IV. Conclusions **27**

Implications for Policy and Practice **23**

Implications for Further Research **33**

Conclusions **35**

IV. References **35**

IV. Dissemination of Research Findings **35**

Journal articles 42

Seminar presentations 42

Poster presentations 43

Professional development 43

Executive Summary

Purpose

Each year in the United States thousands of infants, children, and young adults die suddenly and unexpectedly with no identifiable cause of death. After extensive medicolegal investigation, including autopsy, death certificates variably list cause of death as undetermined, Sudden Infant Death Syndrome (SIDS) (< 1 year old), or Sudden Unexplained Death (SUD) (1-<40years old)(Liberthson 1996; Matthews 2013). The biological mechanisms leading to sudden death (SD) of the young are often unclear. Several exogenous and intrinsic risk factors have been suggested in the pathophysiology of SIDS and SUD (Shephard and Semsarian 2009; Trachtenberg et al. 2012); however, research suggests specific genetic variations underlie the susceptibility of at least some of these individuals to SD (Van Norstrand and Ackerman 2010).

Up to 15% of SIDS and 35% of SUD cases have been estimated to be due to genetic variations in cardiac channel-associated genes (Ackerman 2005; Ackerman et al. 2011; Tester et al. 2012). Therefore, elucidation of the genetic variations involved in SD cases is important to not only establish cause and manner of death of these individuals, but to also aid in determining whether familial genetic testing should be considered. Postmortem genetic screening has been recommended as a new best practice standard in some autopsy-negative cases, however, due to low yields, postmortem genetic screening has not been cost-effective (Skinner et al. 2008; Basso 2010; Ackerman et al. 2011). Due to technological and financial challenges, postmortem genetic screening tools are not currently a feasible option in most medical examiner's and coroner's offices as a standard autopsy procedure.

The purpose of this study was to validate and implement a cost-effective molecular autopsy at the Harris County Institute of Forensic Sciences (Houston, Texas), the county medical

examiner's office for Harris County, Texas, to investigate sudden unexplained deaths of infants and young people. A crucially important and unique aspect of this approach was the development of a multidisciplinary and multi-institutional panel of experts with expertise in clinical and basic science cardiology, genetics and pathology to review each case for putatively significant genetic variants. We hypothesized the postmortem genetic screening method and critical assessment described here could serve as a model for other death investigators considering adopting feasible molecular testing in cases of undetermined cause of death.

Research Design

Autopsy reports of young adults (≤40 years), children, and infants (≤1 year) from 2004-2012 at the Harris County Institute of Forensic Sciences were reviewed, and cases classified as "undetermined", "SIDS", or "undetermined (co-sleeping)" were culled for further analysis. An initial heterogeneous cohort of 429 decedents was defined by medical examiners for postmortem genetic screening. Genomic DNA from the selected cohort was isolated from extracted blood spots previously dried on Whatman FTA® bloodstain cards.

Multiple genes associated with SD due to arrhythmogenic channelopathies and other non-channelopathy disorders were chosen based on literature review and database entries. The 64 selected genes included 22 associated with known cardiac channelopathies, 29 associated with cardiomyopathies, and 13 genes linked to SD without a reported association with a cardiac condition. A library of capture array probes (NimbleGEN SeqCap EZ Choice Library, Roche, Madison, WI) was designed across 94% of the targeted nucleotides (337 kb). The probes were used to create a custom human exonic capture array which was assessed and validated using 24 Coriell HapMap samples.

Sample DNA from the selected cohort was fragmented, prepped, and barcoded to construct pre-capture libraries of DNA that were pooled in equimolar amounts. These library pools were then hybridized in solution to the custom NimbleGen capture probes targeting exonic regions of the genes of interest. Targeted areas were amplified by ligation-mediated PCR, and sequenced using next-generation techniques on an Illumina MiSeq platform. Sequences were mapped to the GRCh37 Human reference genome, and the resulting BAM (binary alignment/map) files were used by ATLAS 2 suite software to call single nucleotide variants (SNVs) and insertion / deletion (indel) variants. These variants were annotated for analysis by Cassandra pipeline software.

De-multiplexed and annotated variants were sorted, compiled in Excel spreadsheets, and culled for possible variants of significance based on initial inclusion criteria of nonsynonymous SNVs and indel variants in exon coding regions followed by comparisons to literature and multiple variant databases. Putative pathogenic variations were further assessed by the Medical Genetics Laboratories at Baylor College of Medicine (BCM-MGL), a Clinical Laboratory Improvement Amendments (CLIA)—accredited laboratory, by American College of Medical Genetic and Genomics (ACMG) certified molecular geneticists (Richards et al. 2008). These variant sequences were orthogonally confirmed by standard Sanger sequencing methods and clinically evaluated for reporting purposes.

A consortium of medical examiners, physicians, and researchers, including specialists in health policy and ethics, reviewed all cases sent for CLIA-laboratory evaluation in preparation for reporting genetic findings that are likely related to death to next-of-kin and supplement the respective autopsy reports. The multidisciplinary board made final diagnostic decisions of the clinical significance of each genetic variant as to cause of death. Classification was based on the

variant evaluation by BCM-MGL, autopsy findings, and available personal and family medical history, and terminal circumstances on a case-by-case basis. Pathogenic genetic defects were sequence variants previously reported and recognized as causative for a specific disorder leading to SD (Richards et al. 2008; Landrum et al. 2014). Incidental or VUS genetic variants were either benign or lacked sufficient evidence to assign causality. Only variants determined to be pathogenic or likely pathogenic were identified as reportable to families. Next-of-kin to decedents received a letter indicating either the genetic change was "likely related to cause of death" (pathogenic) or the genetic change "may or may not be related to cause death" (likely pathogenic).

Findings

The majority of decedents in the cohort were under one year of age (80.7 %). Within this group, the average age was 2.8 months old (± 2.2 months) with 1.5 and 1.8 fold more black infants than Hispanic and white infants, respectively. SUD decedents ranged in age from 1 to 37 years with an average age of 17.6 years old (± 12.1 years). Ethnicity of the older age group was similar for the three primary racial / ethnic groups of Harris County, Texas. The incidence of SD in males was 1.5 times greater than females in both age groups.

Of the 429 individuals initially selected for testing, 351 (280 infants; 71 >1 year of age) had DNA of sufficient quality and quantity for sequencing. A total of 9,318 exonic non-synonymous or insertion/deletion variant (indel) variants were observed in 348 individuals. Tolerated common polymorphisms or variants with no reference data or literature were filtered out. Of the remaining 1,087 total variants, the same genetic variations occurred in multiple cases. In total, 77 unique single nucleotide variants (SNVs) in 29 genes. These variants, plus

one indel, were further assessed for clinical review. Thirteen decedents were found to have reportable (defined as likely related to the cause of death) pathogenic genetic variants. These cases represent 3.7% of the total cohort that was successfully sequenced. Within the specific age groups, 2.8% of the infants (< 1 year of age) and 7.0% of the children/young adults were found to have pathogenic variants associated with cause of death.

The next-of-kin of the decedents found to have a pathogenic variation were notified by mail of the possible amendment to the original autopsy report. In all, seven families responded to the letter sent by the Harris County Institute of Forensic Sciences. Six of seven chose to receive the genetic results which were disseminated by the medical examiner in the presence of a genetic counselor.

Conclusions

Medical examiners have the legal responsibility to identify the cause of death. Underlying genetic variants may be suspected; however, advancements in genetic sequencing have only recently opened the door to accessible genetic screenings for victims of SIDS/SUD. No less important is the ability to reduce the risk of potential criminal investigations of those families affected by SDs of infants or young people.

Molecular autopsies in cases of SD are not a new idea (Tester and Ackerman 2006). Genetic screenings performed as part of the epidemiological assessment in SD cases have been recommended by panels of experts for nearly a decade (Skinner et al. 2008; Basso 2010; Ackerman et al. 2011). However, previous studies have been primarily restricted to small cohorts with a limited number of genes tested (Wong and Behr 2014). These types of assessments have also been cost prohibitive for use in medico-legal investigations. The work

presented here highlights a new transition between research and specific clinical cases to implementation of the molecular autopsy as a cost-effective standard of care in postmortem examinations. By targeting selected exons, cost was kept below $600 per sample. These costs are substantially lower than other available options and are economically feasible for autopsies.

An unexpected finding of this study is the low percentage of individuals found to have a pathogenic variation compared to previous reports (Ackerman 2005; Ackerman et al. 2011; Tester et al. 2012) . Of the total sequenced cohort, less than 4.0% of unexpected deaths were likely due to a pathogenic genetic variant, while published reports have estimated up to 15% of SIDS and 35% of SUD deaths were directly related to specific genetic variations. The difference between these findings and previous reports concerning the prevalence of causal genetic variants is not likely due to intrinsic differences in the cohort, as cohort demographics were consistent with those observed in previous studies. Furthermore, this study represents, to our knowledge, the largest heterogenous cohort tested in a SD postmortem screening.

The low percentage of individuals found to have a pathogenic genetic variant may be the result of the targeted screening approach, as opposed to whole exome or whole genome screening, and differences in variant classification strategies. In this study we incorporated many genes previously included in separate disease-specific assays into one comprehensive genetic screening panel. However, even though this assay is larger than previous genetic panel screens, a drawback to targeting specific genes is that we are limited in the breadth of exome coverage. By not examining the full exome or genome, we likely miss potentially deleterious genetic variations in non-targeted coding or regulatory intronic regions. However, by using a gene targeting approach, we are able to generate a cost-effective molecular autopsy without the burden of massive data sets. In regards to variant classification, the decision to include or exclude a

variant as reportable as contributing to cause of death was based on the strength of literature reports, functional studies, and population frequency data. These discrepancies on the prevalence of pathogenic variant genes linked to SD more likely arise from differences in interpretation of genetic testing results, and the weight given to specific data variables; all influenced by the overarching goal of the individual studies. Total yields may fluctuate using a different variant classification system depending on investigative criteria. As the goal of this study was to describe a feasible manner in which to identify pathogenic variants as part of death investigation, a drawback to this approach is the limitation on the discovery of novel variants, thus reducing total yield.

Multiple factors are considered when determining the potential pathogenicity of a genetic variant. Cosegregation within a family can provide evidence of a heritable genetic defect, while incomplete penetrance in unaffected relatives of a proband warrants critical evaluation to identify a true pathogenic variant. *De novo* variants may provide strong evidence of causation depending on the rarity in the general population. Previous reports have suggested more severe disease expression and earlier onset in *de novo* carriers (Giudici 2014). However, with an extremely rare variant, a lack of further *in vitro* / *in vivo* functional evidence and clinical reports may lead to a categorization of a putative pathogenic variant as a VUS (Dorschner et al. 2013; Tang et al. 2014). Final determinations of cause of death due to the expression of specific genetic variants were concluded by an expert panel based on genetic screening results, autopsy findings, sentinel events, and personal and family medical histories.

The wider implementation of molecular autopsy tools, such as the one presented here, can aid in bringing clarity to these ambiguous molecular pathways and help to identify true pathogenic variants. However, agencies need to be cognizant of the ethical implications of

molecular genetic testing and reporting. In some cases disclosure of sensitive genetic results may lead to the discovery of unwanted information, increased insurance burdens, unnecessary medical testing, or altered family planning (Ross et al. 2013; Clarke 2014). The results of these molecular autopsies are envisioned to become part of the normal medicolegal death investigation, and, will in some instances result in supplements or amendments of autopsy reports and death certificates. These challenges emphasize the need for a critical evaluation of genetic defects to help establish proper evidentiary thresholds for reporting as contributing factors to cause of death.

In conclusion, the present study represents the largest heterogeneous cohort of SD cases evaluated by a large targeted sequencing panel for genes associated with SIDS and SUD. This genetic screening strategy offers a cost-effective tool for medical examiners for diagnosis in autopsy negative cases of SD in the young. In addition to being diagnostic in nature, these screenings allow for affected families to make appropriate choices regarding medical testing, treatment, and lifestyle. The work presented here demonstrates the necessity to establish an interdisciplinary program for genetic result interpretation, disclosure, and follow-up counseling that accompanies the implementation of postmortem genetic screening. As the dynamic field of molecular diagnostics advances, molecular autopsy tools have begun to be within reach as a new standard of care for autopsy negative cases.

I. Introduction

Each year in the United States thousands of infants, children, and young adults die suddenly and unexpectedly with no identifiable cause of death. After extensive medicolegal investigation, including autopsy, death certificates variably list cause of death as undetermined, Sudden Infant Death Syndrome (SIDS) (< 1 year old), or Sudden Unexplained Death (SUD) (1-<40years old)(Liberthson 1996; Matthews 2013). The biological mechanisms leading to sudden death (SD) of the young are often unclear. Several exogenous and intrinsic risk factors have been suggested in the pathophysiology of SIDS and SUD (Shephard and Semsarian 2009; Trachtenberg et al. 2012); however, research suggests specific genetic variations underlie the susceptibility of at least some of these individuals to SD (Van Norstrand and Ackerman 2010).

For young people, sudden unexpected death is most often suspected to be of cardiac origin, or sudden cardiac death (SCD). SCD can be described as a natural death caused by sudden arrhythmia often occurring without previous warning or symptoms (Shephard and Semsarian 2009). In many of these cases structural abnormalities of the heart, cardiomyopathies, are evident at autopsy. Cardiomyopathies, such as hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DC), have been associated with variations in genes that encode cardiac structural proteins. In cases of SD where no structural cardiovascular or other anatomical abnormalities are observed at autopsy, a fatal arrhythmogenic disorder may be suspected because of clinical, historical, or circumstantial information; however, due to a negative autopsy exam the cause of death is left as undetermined (Shephard and Semsarian 2009).

Arrhythmogenic disorders such as Long QT Syndrome (LQTS), Short QT Syndrome (SQTS), Brugada Syndrome (BRs) and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) are characterized by electrical disturbances in heart function, unaccompanied by

anatomic evidence. These disorders, also known as channelopathies, are linked to putative pathogenic variations in the genes that encode for cardiac ion channels or ion channel-associated proteins (Tester and Ackerman 2009). Up to 15% of SIDS and 35% of SUD cases have been estimated to be due to genetic variations in cardiac channel-associated genes (Ackerman 2005; Ackerman et al. 2011; Tester et al. 2012).

In many case reports and clinical evaluations where genetic variants or inherited cardiac disease have been associated with a sudden death, some victims are speculated to have *de novo* pathological variants with SD as the sentinel event (Klaver et al. 2011; Tester et al. 2012; Giudici 2014). However, a variety of reports indicate that many of the known genetic variations underlying SCD due to either cardiomyopathy or channelopathy are autosomal dominant and have a 50% chance of inheritance (Shim 2005; Shephard and Semsarian 2009). Therefore, elucidation of the genetic mutations involved in SD cases is important not only to establish cause and manner of death of these individuals, but also to aid in determining whether familial genetic testing should be considered. Postmortem genetic screening has been recommended as a new best practice standard in some autopsy-negative cases, however, due to low yields, postmortem genetic screening has not been cost-effective (Skinner et al. 2008; Basso 2010; Ackerman et al. 2011). An international heart rhythm expert panel further recommended mutation-specific genetic testing and clinical screenings for family members of some sudden death decedents (Ackerman et al. 2011). Due to technological and financial challenges, postmortem genetic screening tools are not currently a feasible option in most medical examiner's and coroner's offices as a standard autopsy procedure.

Commercial genotyping tests are available to identify several genetic variants putatively linked to SD. Labor intensive Sanger sequencing methods for the identification of disease-

specific genetic variants can cost $5400 or more per case to sequence fewer than twenty genes (Bai et al. 2009). Faster and more comprehensive whole genome or exon-specific analysis using next-generation sequencing (NGS) techniques are 20-fold less expensive than Sanger sequencing methods, however are not yet economically feasible for routine use by public service agencies (Loporcaro et al. 2014; Wetterstrand 2014).

The purpose of this study was to validate and implement a cost-effective molecular autopsy at the Harris County Institute of Forensic Sciences (Houston, Texas), the county medical examiner's office for Harris County, Texas, to investigate sudden unexplained deaths of infants and young people. A cohort of 351 young individuals who died of sudden and mostly unexplained causes was tested for determination of cause of death. When this project was originally purposed, the goal was to sequence exonic regions of 27 genes linked to sudden cardiac arrhythmic death for less than $1000 per individual. However, during the course of this study, the goal was updated to 64 genes, including genes implicated in non-cardiac SD pathology. Increasing the number of genes tested added some additional interpretation time, however, the larger DNA target amounts served to improve efficiency of gene targeting without an additional cost per individual. Using a custom gene target exon capture array, the genes were assessed by NGS technology (Table 1; Supplemental Table 1).

Table 1. Targeted genes.

Cardiac Channelopathy / Arrhythmia Associated Genes			Cardiomyopathy Associated Genes			Non-Cardiac Associated Genes	
AKAP9	*KCNE1*	*RYR2*	*ACTC1*	*LDB3*	*RAF1*	*ACADM*	*KCNQ2*
ALG10B	*KCNE2*	*SCN10A*	*ANKRD1*	*LMNA*	*TCAP*	*IL10*	*KCNQ3*
ANK2	*KCNE3*	*SCN1B*	*DSC2*	*MYBPC3*	*TGFB3*	*IL1A*	*PYGM*
CACNA1C	*KCNH2*	*SCN3B*	*DSG2*	*MYH7*	*TMEM42*	*IL1B*	*SCN2A*
CACNB2	*KCNJ2*	*SCN4B*	*DSP*	*MYH7B*	*TNNC1*	*IL6*	*SL37A4*
CASQ2	*KCNQ1*	*SCN5A*	*FXN*	*MYL2*	*TNNI3*	*KCNA1*	*SLC6A4*
CAV3	*NOS1AP*	*SNTA1*	*GLA*	*MYL3*	*TNNT2*	*TNF*	
GPD1L			*JPH2*	*PKP2*	*TPM1*		
			JUP	*PLN*	*VCL*		
			LAMP2	*PRKAG2*			

A crucially important and unique aspect of this approach was the development of a multidisciplinary and multi-institutional panel of experts with expertise in clinical and basic science cardiology, genetics and pathology to review each case for putatively significant genetic variants. The panel assessed each mutation in light of the existing literature for biochemical and functional mutational analysis, known allele frequency, and case history to determine the likelihood of causality of each sequence variant in SD. Genetic counselors were also enlisted to review the variants thus identified and to be available for dissemination of results to surviving family members in an ethical and accurate manner.

To our knowledge, this study is the largest cohort to date screened by NGS targeted to genes known to underlie SD and performed as part of medicolegal investigations of sudden unexplained death cases. The use of a multidisciplinary consortium to assess likelihood of relevance for each mutation can serve as a model for other death investigators considering adopting molecular testing. At ~$600 per case, this low cost SD molecular autopsy has the potential to aid in determination of cause and manner of death in many unexplained cases in

Harris County, Texas, each year; moreover, this approach to data analysis can also be adopted by other medical examiner offices as NGS becomes more widely available.

II. Methods

Cohort

Autopsy reports of young adults (≤40 years), children, and infants (≤1 year) from 2004-2012 at the Harris County Institute of Forensic Sciences were reviewed, and cases classified as "undetermined", "SIDS", or "undetermined (co-sleeping)" were culled for further analysis. Any case with indicators of non-natural etiology including cases with any suspicion of death by an inflicted mechanism (e.g. suspicion of intentional suffocation) was excluded. An initial heterogeneous cohort of 429 decedents was defined by medical examiners for postmortem genetic screening. Also included in the cohort were a few cases that had nonspecific anatomic cardiac changes suggesting a manifestation of an underlying genetic defect (i.e., borderline cardiomyopathy), one case with a listed cause of death as hypertrophic cardiomyopathy, and an additional case with previous medical history suggesting LQTS. These cases were included in order to obtain genetic confirmation and/or specific diagnosis of a disorder where anatomic findings were equivocal. In addition to the genetic screenings, the demographic composition of the selected cohort was compared to age-matched populations in Harris County, Texas to identify possible at-risk groups.

DNA extraction

Total DNA was extracted from archived blood spots dried on Whatman® bloodstain cards (WB100014) from the selected cohort using a QIAsymphony DSP DNA Midi Kit (Qiagen, Germantown, Maryland) per manufacturer protocol. Briefly, bloodstain card cuttings (~3 cm^2)

were incubated in 1 ml of Buffer ATL (Qiagen, Germantown, Maryland) containing 1 mg/ml Proteinase K (Qiagen, Germantown, Maryland) at 56ºC with shaking at 900 RPM for eight hours. Genomic DNA was isolated from the lysates on a QIAsymphony SP instrument and eluted at 100 µl per sample.

Capture array design and validation

Multiple genes associated with SD due to arrhythmogenic channelopathies and other non-channelopathy disorders were chosen based on literature review and database entries. The 64 selected genes included 22 associated with known cardiac channelopathies, 29 associated with cardiomyopathies, and 13 genes linked to SD without a reported association with a cardiac condition (Table 1). A library of capture array probes (NimbleGEN SeqCap EZ Choice Library, Roche, Madison, WI) was designed across 94% of the targeted nucleotides (337 kb). The applicable genome coordinates from the human genome build hg19 were obtained from the UCSC Genome Database (*http://genome.ucsc.edu/*) and submitted for capture probe design by Roche NimbleGEN. The probes were used to create a custom human exonic capture array which was assessed and validated by the Baylor College of Medicine Human Genome Sequencing Center (Houston, Texas) using 24 Coriell HapMap samples. The resulting capture reagent was found to yield 91.2% of targeted bases covered at 20x or better. After validation, the capture array was utilized to enrich the targeted gene regions of the cohort DNA samples in preparation for high throughput parallel next-generation sequencing.

Sample entry, library preparation, targeted capture, and sequencing

Prior to target capture, DNA samples were tested for quality and quantity by a combination of agarose gel electrophoresis and PicoGreen fluorescence on an Agilent Bioanalyzer 2100. Samples passing minimum standards were used to construct Illumina paired-

end pre-capture libraries according to the manufacturer's protocol (*Illumina Multiplexing_SamplePrep_Guide_1005361_D*) with modifications as described in the BCM-HGSC protocol (https://www.hgsc.bcm.edu/content/protocols-sequencing-library-construction). BCM-HGSC exome capture methods, adapted from the manufacturer's protocol (*NimbleGen SeqCap EZ Exome Library SR User's Guide Version 2.2*), were further modified for targeted capture to allow for increased multiplexing in capture and sequencing. Briefly, 1 µg of sample DNA was sheared into fragments of approximately 300-400 base pairs using a Covaris E210 system. End-repair, A-tailing, ligation of 9bp barcode adaptors (24 barcodes in total), pre-capture ligation-mediated PCR (LM-PCR), as well as the SPRI bead purification (Agencourt AMPure XP beads) was automated on Biomek FXp robotic workstations (Adey et al. 2010). Uniquely barcoded pre-capture libraries were pooled in equimolar amounts (24 samples/pool, totaling 1ug/pool) for co-capture. These library pools were then hybridized in solution to the custom NimbleGen capture design in the presence human COT1 DNA to suppress repetitive genomic sequences. After post-capture LM-PCR amplification and a final SPRI bead purification, pooled samples were loaded on an Illumina MiSeq instrument for cluster formation. Library templates underwent bridge amplification to form clonal clusters followed by hybridization with the sequencing primer. Sequencing runs were also performed on the Illumina MiSeq platform in paired-end mode where sequencing-by-synthesis reactions were extended for 101 cycles from each end, with an additional 10 cycles for the index read. Sequencing runs yielded an average of ~79.4 Mb per sample and an average of 85.3% of the targeted bases covered to a depth of 20X or greater.

Primary data analysis

Initial sequence analysis was performed using the HGSC Mercury analysis pipeline (https://www.hgsc.bcm.edu/content/mercury) (Reid et al. 2014). Briefly, the .bcl files produced on-instrument were first transferred into the HGSC analysis infrastructure by the HiSeq Real-time Analysis module and Mercury then subjected to CASAVA, the primary analysis software, in order to de-multiplex pooled samples, and generate sequence reads and base-call confidence values (qualities). Reads were then mapped to the GRCh37 Human reference genome (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/) using the Burrows-Wheeler aligner (BWA, http://bio-bwa.sourceforge.net/) (Li and Durbin 2009). The resulting BAM (binary alignment/map) file underwent quality recalibration using GATK (http://www.broadinstitute.org/gatk/) for BAM sorting, duplicate read marking, and realignment to improve indel discovery (Li et al. 2009; DePristo et al. 2011). Finally, BAM files were used by the ATLAS 2 suite (https://www.hgsc.bcm.edu/software/atlas-2) to call SNVs and indel variants and to produce vcf files with variant annotation provided by the Cassandra pipeline (https://www.hgsc.bcm.edu/software/cassandra) (Challis et al. 2012).

Variant filtration

De-multiplexed and annotated variants were initially sorted by sequence quality. Variant sequences not meeting a computational "Pass" threshold for quality sequence coverage were excluded. Quality annotated variants were compiled in Excel spreadsheets and culled for possible mutations of significance based on variant type with initial inclusion criteria of nonsynonymous SNVs and indel variants in exon coding regions (Figure 1). Candidate variants were further assessed by comparison to literature and databases searches via Single Nucleotide

Polymorphism Database (dbSNP)(Sherry et al. 2001), Online Mendelian Inheritance in Man database (OMIM)(http://www.ncbi.nlm.nih.gov/omim), and The Inherited Arrhythmias Database (www.fsm.it/cardmoc/). Variants with conflicting, minimal or no literature support, those identified in single individuals, or variants of unknown significance were removed from further analysis.

Figure 1.

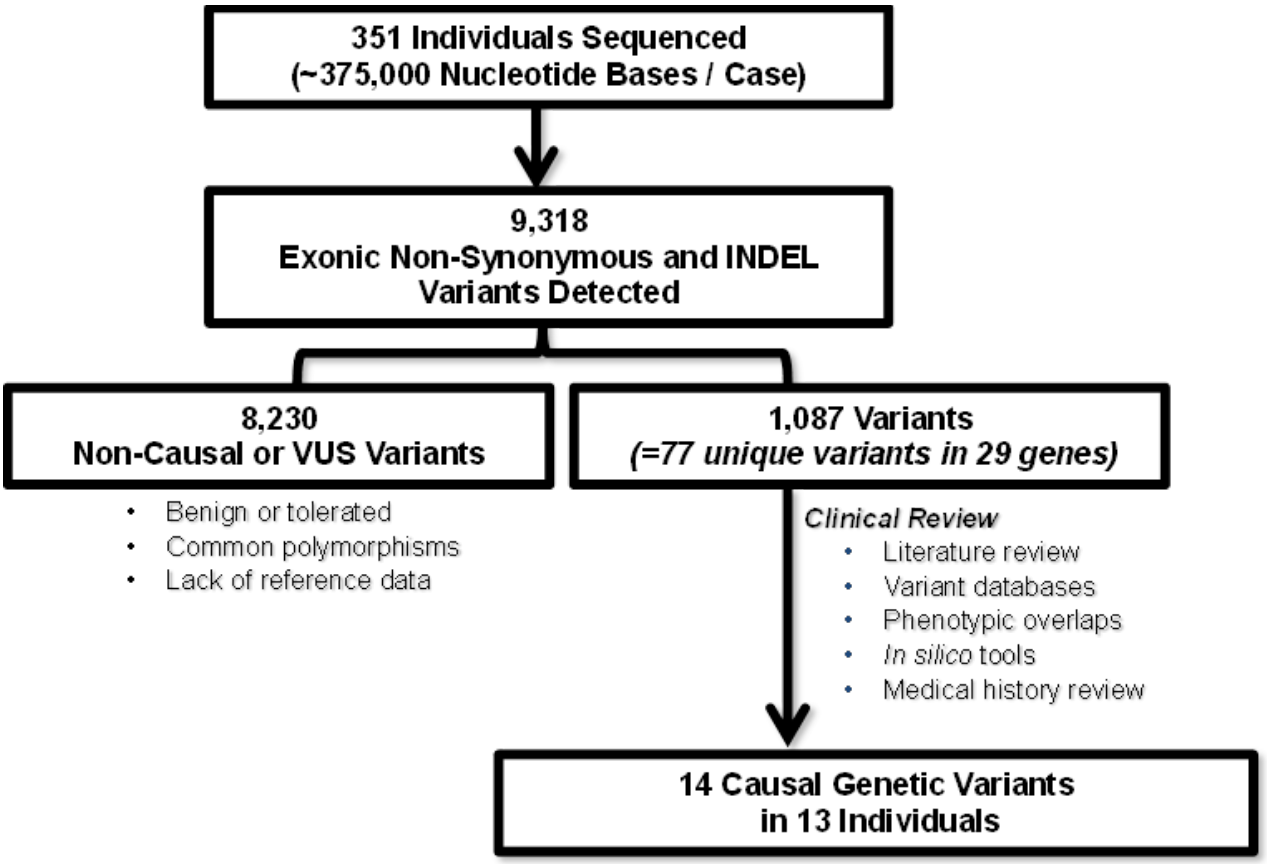

Figure 1. Workflow of genetic variant screening. All quality annotated SNVs and indel variants were filtered in order to classify variants as non-causal or causal in SD.

The putative pathogenic variations were further assessed by the Medical Genetics Laboratories at Baylor College of Medicine (BCM-MGL), a Clinical Laboratory Improvement Amendments (CLIA)—accredited laboratory, by American College of Medical Genetic and Genomics (ACMG) certified molecular geneticists (Richards et al. 2008). Variant sequences were orthogonally confirmed by standard Sanger sequencing methods and clinically evaluated for reporting purposes. Briefly, the phenotypic overlap between the particular pathogenic variant and the associated disease were compared to the phenotype of the decedent. Most variants not consistent with autopsy findings were then eliminated. Once the phenotype-genotype correlation was established, the inheritance pattern was evaluated. For example, in cases of autosomal recessive disorders, two pathogenic changes needed to be detected in a particular gene. Biochemical phenotypes and minor allele frequencies for specific ethnic groups were assessed for each variant by comparison to the single nucleotide polymorphism database (dbSNP), the Exome Variant Server (http://evs.gs.washington.edu/EVS/), ClinVar, the 1000 Genomes Project Browser, Baylor College of Medicine Medical Genetics Laboratory database and the Human Gene Mutation Database (McVean et al. 2012; Stenson et al. 2014). *In silico* tools including Polymorphism Phenotyping v2 (PolyPhen-2), Sorts Intolerant From Tolerant Substitutions tool (SIFT), and Protein Variation Effect Analyzer (PROVEAN) algorithmic tools to predict deleterious effects of mutation-induced structural changes on protein function were also considered during variant evaluation (Ng and Henikoff 2001; Adzhubei et al. 2010; Choi et al. 2012). Extensive evaluations of published literature and comparisons to previously reported genotype / phenotype relationships for each sequence-confirmed putative pathogenic variant were carried out to determine the functional significance of the genetic mutation (Landrum et al. 2014).

Clinical genetic variant confirmation

A consortium of medical examiners, physicians, and researchers, including specialists in health policy and ethics, reviewed all cases sent for CLIA-laboratory evaluation in preparation for reporting genetic findings that are likely related to death to next-of-kin and supplement the respective autopsy reports. The multidisciplinary board made final diagnostic decisions of the clinical significance of each genetic variant as to cause of death. Variants were classified as: 1) pathogenic (most likely related to cause and manner of death), 2) likely pathogenic (significant but not conclusively related to cause of death), or 3) Incidental or VUS (not related or uncertain clinical significance). Classification was based on the variant evaluation by BCM-MGL, autopsy findings, and available personal and family medical history, and terminal circumstances on a case-by-case basis. Pathogenic genetic defects were sequence variants previously reported and recognized as causative for a specific disorder leading to SD (Richards et al. 2008; Landrum et al. 2014). Incidental or VUS genetic variants were either benign or lacked sufficient evidence to assign causality.

Only variants determined to be pathogenic or likely pathogenic were identified as reportable to families. Next-of-kin to decedents received a letter indicating either the genetic change was "likely related to cause of death" (pathogenic) or the genetic change "may or may not be related to cause death" (likely pathogenic). Families were given a choice to receive further information. If those families chose to receive the results, the information was disseminated by the medical examiner in the presence of a genetic counselor.

III. Results

Cohort demographics

Harris County, Texas, is the third most populous county in the United States (United States Census Bureau (http://quickfacts.census.gov/qfd/states/48/48201.html). As of the 2010 national census, the primary racial groups within the county include Hispanic or Latino (41.6%), Non-Hispanic or Latino White (31.9%), Non-Hispanic Black or African American (19.5%), and Asian (6.8%). In comparison, the demographic characteristics of the Harris County Institute of Forensic Sciences SIDS/SUD cohort selected for this study are described in Table 2.

Table 2. Cohort demographics.

Age Group:	**0-12 Months (*n*= 346)**		**1-40 years (*n*=83)**	
	Characteristic	**Number of cases, (% of *n*)**	**Characteristic**	**Number of cases, (% of *n*)**
Sex	**Male**	208 (60.1%)	**Male**	52 (62.7%)
	Female	138 (39.9%)	**Female**	31 (37.3%)
Ethnicity (Race/Hispanic origin)*	**Black**	155 (45.9%)	**Black**	27 (30.3%)
	Hispanic	101 (29.7%)	**Hispanic**	27 (30.3%)
	White	86 (25.3%)	**White**	22 (24.7%)
	Asian	4 (1.2%)	**Asian**	2 (2.2%)
	Other	0	**Other**	5 (5.6%)

*Race is of non-Hispanic origin unless otherwise indicated

The majority of decedents in the cohort were under one year of age (80.7 %). Within this group, the average age was 2.8 months old (± 2.2 months) (Figure 1) with 1.5 and 1.8 fold more black infants than Hispanic and white infants, respectively (Table 2). SUD decedents ranged in age from 1 to 37 years with an average age of 17.6 years old (± 12.1 years) (Figure 2). Ethnicity of the older age group was similar for the three primary racial / ethnic groups of Harris County, Texas. The incidence of SD in males was 1.5 times greater than females in both age groups.

Figure 2.

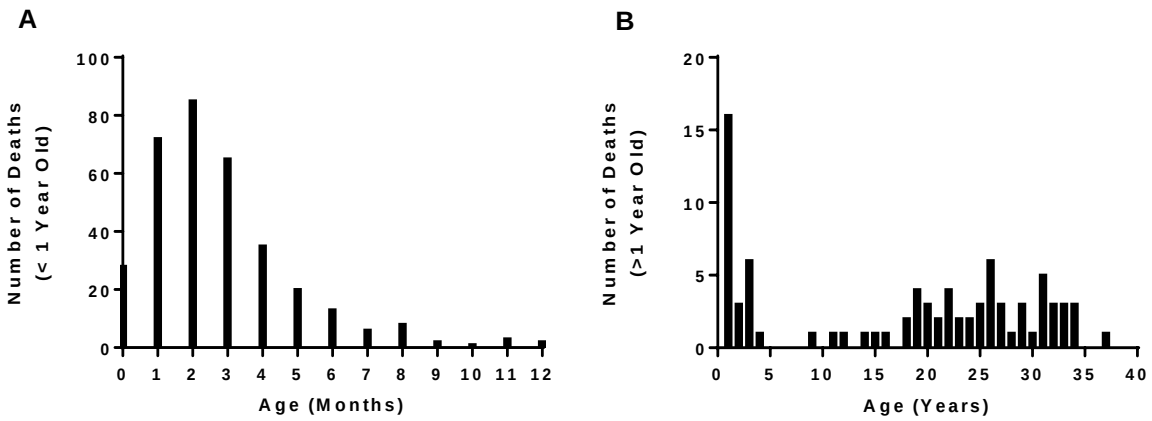

Figure 2. Sudden death age distribution in the Harris County Institute of Forensic Sciences SIDS/SUD cohort (2004-2012). **A**. Age distribution of SIDS victims (0-12 months, *n*=346). The majority of the unexplained deaths decedents in the cohort were under the age of one. Distribution of age was unimodal, skewed right, with the highest frequency of death occurring at 2 months old (95% CI: 2.6 to 3.0 months; Bin range = 1 Month). **B**. Age distribution of SUD victims (1-40 years, *n*=83). The distribution of age was random for unexplained death decedents, with the highest frequency of occurrence in young children between 1-2 years of age (95% CI: 15.0 to 20.3 years; Bin range = 1 Year). Note the five-fold Y-axis scale difference between A and B.

Genetic variants

Of the 429 individuals initially selected for testing (Table 2), 351 (280 infants; 71 >1 year of age) had DNA of sufficient quality and quantity for sequencing. After primary quality data analysis with comparisons to the GRCh37 Human reference genome, a total of 9,318 exonic non-synonymous or indel variants were observed in 348 individuals. Tolerated common polymorphisms or variants with no reference data or literature were filtered out (Figure 1). Of the remaining 1,087 total variants, the same genetic variations occurred in multiple cases

(Supplemental Table 2). In total, 77 unique single nucleotide variants (SNVs) in 29 genes. These variants, plus one insertion/deletion variant (indel), were further assessed for clinical review. Thirteen decedents were found to have reportable (defined as likely related to the cause of death) pathogenic genetic variants (Tables 3 and 4). One individual had two genetic variants likely leading to SD. These cases represent 3.7% of the total cohort that was successfully sequenced. Within the specific age groups, 2.8% of the infants (< 1 year of age) and 7.0% of the children/young adults were found to have pathogenic variants associated with cause of death. The reportable variants were all single base changes. Additionally, of the 64 genes in the 351 individuals tested, a total of 9 genes were found to have pathogenic variations in the thirteen reported decedents. Causal genetic variants in S*CN3B* and *MYL2* were observed twice in these individuals, while variants in *SCN5A* were observed four times.

Table 3. Infant (0-12 Months) cohort with confirmed causal genetic variations leading to sudden death.

Case	Age	Sex	Ethnicity[a]	Gene(s)	Nucleotide Change(s)	Amino Acid Change(s)	Listed Cause / Manner of Death[b]
1	1 Month	M	Black	*SCN3B*	G328A	V110I	Undetermined / Undetermined
2	2 Months	F	Black	*ANK2*	C5461T	R1821W (R1788W)	Undetermined / Undetermined
3	3 Months	M	White	*SCN5A*	G1844A	G615E	SIDS / Undetermined
4	3 Months	M	White	*MYL2*	C141A	N47K	SIDS / Natural
5	5 Months	M	Hispanic White	*SCN5A*	G80A	R27H	SIDS / Natural
6	5 Months	M	Hispanic White	*RYR2*	C3320T	T1107M	SIDS / Natural
7	5 Months	F	Black	*SCN5A*	C5549T	S1904L	SIDS / Natural
8	6 Months	M	Hispanic White	*GPD1L*	C839T	A280V	Undetermined / Undetermined

[a]Ethnicity refers to observed race and Hispanic origin. Race is of non-Hispanic origin unless otherwise indicated.
[b]Cause and manner of death as listed in original Harris County Institute of Forensic Sciences autopsy report.

Table 4. Children/young adult (1-40 years) cohort with confirmed causal genetic variations leading to sudden death.

Case	Age	Sex	Ethnicity[a]	Gene(s)	Nucleotide Change(s)	Amino Acid Change(s)	Listed Cause / Manner of Death[b]
9	15 Years	M	White	*DSP*	G88A	V30M	Hypertrophic cardiomyopathy/ Natural
10	24 Years	F	White	*SCN3B*	C423G	I141M	Long QT syndrome/ Natural
11	27 Years	M	Hispanic White	*MYL2*	G64A	E22K	Undetermined / Undetermined
12	32 Years	F	White	*SCN5A; SCN1B*	G1844A; G457A	G615E; D153N	Undetermined / Undetermined
13	37 Years	M	White	*MYBPC3*	G13C	G5R	Cardiac arrhythmia associated with left ventricular hypertrophy / Natural

[a]Ethnicity refers to observed race and Hispanic origin. Race is of non-Hispanic origin unless otherwise indicated.
[b]Cause and manner of death as listed in original Harris County Institute of Forensic Sciences autopsy report.

The next-of-kin of the decedents found to have a pathogenic variation were notified by mail of the possible amendment to the original autopsy report. In all, seven families responded to the letter sent by the Harris County Institute of Forensic Sciences. Six of seven chose to receive the genetic results which were disseminated by the medical examiner in the presence of a genetic counselor.

Data access

All non-synonymous variants are listed in Supplemental Table 2. Confirmed pathogenic genetic variant data have been submitted to ClinVar under accession numbers SCV000263109, SCV000263110, SCV000263111, SCV000263112, SCV000263113 SCV000263114, SCV000263115, SCV000263116, SCV000263117, SCV000263118, SCV000263119, SCV000263120, SCV000263121, and SCV000263122. The full sequencing data were collected as continuation of medico-legal investigation, and therefore these data can be made available upon request to the Harris County Institute of Forensic Sciences.

IV. Conclusions

Discussion of Findings

The sudden and unexpected death of an infant or young family member has devastating effects on the family and community (Jind et al. 2010). These effects are compounded by the lack of a definitive cause of death. Medical examiners have the legal responsibility to identify the cause of death. Underlying genetic variants may be suspected; however, advancements in genetic sequencing have only recently opened the door to accessible genetic screenings for victims of SIDS/SUD. No less important is the ability to reduce the risk of potential criminal investigations of those families affected by SDs of infants or young people.

Molecular autopsies in cases of SD are not a new idea (Tester and Ackerman 2006). Genetic screenings performed as part of the epidemiological assessment in SD cases have been recommended by panels of experts for nearly a decade (Skinner et al. 2008; Basso 2010; Ackerman et al. 2011). However, previous studies have been primarily restricted to small cohorts with a limited number of genes tested (Wong and Behr 2014). These types of assessments have also been cost prohibitive for use in medico-legal investigations. The work presented here highlights a new transition between research and specific clinical cases to implementation of the molecular autopsy as a cost-effective standard of care in postmortem examinations. By targeting selected exons, cost was kept below $600 per sample (Table 5). These costs are substantially lower than other available options and are economically feasible for autopsies.

Table 5. Sequencing Costs

Item	Description	Price per sample[a]
Extraction Reagents	QIAsymphony DSP Midi Kit	$6.42
NimbleGEN SeqCap Capture Array	Roche – Library Design and Probes	$4.27
Human Genome Sequencing Center – Baylor College of Medicine	Library Preparation Target Capture Illumina MiSeq Sequencing Primary Data Analysis Sequencing Center Labor	$341.63
Medical Genetics Laboratories – Baylor College of Medicine	Clinical verification of variants[b]	$21.65
Post-doctoral Salary	Salary and Benefits (40 Hrs*52 Wks)[c]	$194.13
Total		**$568.10**

[a]Number of samples successfully sequenced (*n*= 351)
[b]Analysis and Sanger sequencing of 38 genetic variants at $200/sample
[c]One post-doctoral researcher - assuming salary and benefits of $68,140 per year
Labor costs of work performed at the Human Genome Sequencing Cener and Medical Genetics Labo is included in the total price per sample

An unexpected finding of this study is the low percentage of individuals found to have a pathogenic variation compared to previous reports (Ackerman 2005; Ackerman et al. 2011; Tester et al. 2012) . Of the total sequenced cohort, less than 4.0% of unexpected deaths were likely due to a pathogenic genetic variant, while published reports have estimated up to 15% of SIDS and 35% of SUD deaths were directly related to specific genetic variations. Discrepancies between previous reports on the prevalence of pathogenic variant genes linked to SD could arise from differences in multiple evaluation techniques such as: 1) cohort composition, 2) genetic screening method, or 3) interpretation of genetic testing results.

First, for this study, a large cohort was selected based on autopsy findings. Cohort demographics were consistent with those observed previously, such as increased incidences of SD reported for males, higher SIDS rates for non-Hispanic black infants compared to Hispanic and non-Hispanic white infants, and the majority of unexpected infant deaths occurred in the youngest of the group (93.5% under 6 months of age) (Shen et al. 1995; Matthews 2013; Wang

et al. 2014). A larger cohort allows for an accurate estimate of the rate of SD of the young in a heterogeneous population that are attributable to causal mutations. Therefore, the difference between these findings and previous reports concerning the prevalence of causal genetic variants is not likely due to intrinsic differences in the cohort evaluated in this study. However, the evaluation of candidate SD cases for genetic screening may differ between medical examiner offices which would alter the initial size and make-up of cohorts. A more restrictive cohort selection, such as phenotype- or familial history-guided testing may increase the overall likelihood of a positive genetic test result. On the other hand, a less restrictive inclusion criterion with an equally critical genetic evaluation could increase the number of decedents evaluated and decrease this overall percentage of a positive finding. Nevertheless, these nuances need to be weighed carefully in discerning the best course of action in development of molecular autopsies for use in death investigations, and may affect the cost and/or overall yield.

In regards to the genetic screening method, we tested a panel of 64 genes associated with multiple diseases linked to SD both from cardiac and non-cardiac causes. Others have reported sequence results for disease-specific genetic panels with smaller cohorts or full exome analysis of specific individuals (Ackerman et al. 2004; Tester et al. 2005; Tester et al. 2012; Papadakis et al. 2013; Brion 2014). In this study we incorporated many genes previously included in separate disease-specific assays into one comprehensive genetic screening panel. A drawback to targeting specific genes is that we are limited in the breadth of exome coverage. By not examining the full exome or genome, we likely miss potentially deleterious genetic variations in non-targeted coding or regulatory intronic regions. However, by using a gene targeting approach, we are able to generate a cost-effective molecular autopsy without the burden of massive data sets.

The low percentage of individuals found to have a pathogenic genetic variant may be the result of differences in variant classification. The decision to include or exclude a variant as reportable as contributing to cause of death was based on strength of literature reports, functional studies, and population frequency data. How this data is weighed and the overarching goal of a study may differ between laboratories resulting in variability of the total yield of genetic variants. This total yield may increase using a different variant classification system depending on investigative criteria. As the goal of this study was to describe a feasible manner in which to identify pathogenic variants as part of death investigation, a drawback to this approach is the limitation on the discovery of novel variants. The identification of novel variants in postmortem genetic screening of large heterogeneous cohorts has been described elsewhere (Wang et al. 2014), and while important and necessary in the sudden death field, it was beyond the scope of this study.

In this report, putative lethal variants were reviewed by certified molecular geneticists using ACMG standards to determine clinical significance on a case-by-case basis. A "variant of unknown significance" (VUS), where there is insufficient evidence to support deleteriousness, was not reported as likely pathogenic or pathogenic. Most of the genetic variants that are known to be involved in SD have incomplete penetrance or variable expressivity (Ackerman et al. 2011). This phenotypic diversity could be due to unidentified differences in environmental triggers, epigenetic modifiers or developmental changes making genetic diagnosis of a disease challenging and easily misinterpreted (Sen-Chowdhry et al. 2010; Giudicessi and Ackerman 2013). As a result of a conservative screening approach in this study, it is likely that novel or VUS genetic variants that were causal in SD were not identified as pathogenic due to a lack of data at the time of review. Overall, these challenges emphasize the need for a critical evaluation

of genetic defects by expert medical geneticists in order to help establish proper evidentiary thresholds for reporting as contributing factors to cause of death.

Multiple factors are considered when determining the potential pathogenicity of a genetic variant. Co-segregation within a family can provide evidence of a heritable genetic defect, while incomplete penetrance in unaffected relatives of a proband warrants critical evaluation to identify a true pathogenic variant. *De novo* variants may provide strong evidence of causation depending on the rarity in the general population. Previous reports have suggested more severe disease expression and earlier onset in *de novo* carriers (Giudici 2014). However, with an extremely rare variant, a lack of further *in vitro* / *in vivo* functional evidence and clinical reports may lead to a categorization of a putative pathogenic variant as a VUS (Dorschner et al. 2013; Tang et al. 2014). Final determinations of cause of death due to the expression of specific genetic variants were concluded by an expert panel based on genetic screening results, autopsy findings, sentinel events, and personal and family medical histories.

The pathogenic genetic variants occurred in a limited set of genes. Of the thirteen SD cases determined to have a causal variant, only nine genes were represented out of the original list of 64 (Table 3 and 4). All pathogenic variants were either channelopathy- or cardiomyopathy-associated genes. Specifically, six cases of SD were caused by sodium channel defects alone. The cases with a specified cause of death of cardiomyopathy were confirmed, and the specific causative genetic defect was identified. However, other decedents with negative autopsies also had variants in heritable cardiomyopathy-associated genes suggesting not all lethal structural protein defects are evident at autopsy thus emphasizing the necessity of diagnostic postmortem genetic screening.

Implications for Policy and Practice

The wider implementation of molecular autopsy tools, such as the one presented here, can aid in bringing clarity to these ambiguous molecular pathways and help to identify true pathogenic variants. This highlights the need for medical examiners to not only maintain archives of blood samples of SIDS/SUD victims, but also detailed case history records and databases of sequencing results for continuous reassessment as new molecular, biochemical, and functional evidence is discovered and gene candidates are further defined.

However, agencies need to be cognizant of the ethical implications of molecular genetic testing and reporting. In some cases disclosure of sensitive genetic results may lead to the discovery of unwanted information, increased insurance burdens, stressful unnecessary medical testing, or altered family planning (Ross et al. 2013; Clarke 2014). Therefore, molecular autopsy programs are best served by the inclusion of experts in biomedical ethics and genetic counselors on review boards. For this study, a consortium of clinical and genetic experts from the Harris County Institute of Forensic Sciences and Baylor College of Medicine collaborated with the Baylor College of Medicine Center for Medical Ethics and Health Policy to establish guidelines on the disclosure of genetic information. Families were given the choice to receive genetic results, and if they chose to receive them, were professionally counseled accordingly. While not included in the overall cost of testing for this report, genetic counseling is advantageous in helping the family of SIDS/SUD victims understand complicated diagnostics and to offer advice on future clinical and genetic testing. The results of these molecular autopsies are envisioned to become part of the normal medicolegal death investigation, and, will in some instances result in amendments of autopsy reports and death certificates.

Implications for Further Research

These results demonstrated a cost-effective strategy for application of molecular autopsies. With the unexpected low yield of reportable pathogenic variants it is conceivable that detection of pathogenic variants may still not be feasible in all medico-legal investigations with an undetermined cause of death finding. This study is limited by its retrospective nature with an additional goal of determining the yield of reportable pathogenic variants using methods presented here. As expected in a large retrospective cohort, some costs would be amortized. However, as costs continue to fall with advancements in mass parallel sequencing, the role of genetic variants in SD will be elucidated further thus possibly increasing the overall benefit of postmortem genetic screening in medico-legal investigations.

Sudden death of the young and SIDS are complex and multifactorial diseases. In many cases, it is likely that multiple molecular mechanisms including causative single gene mutations, weak additive effects of both common and rare genetic variants, gene X environment interactions, inter-current illnesses and medications, underlying comorbidities (e.g. epilepsy) and other intrinsic and extrinsic risk factors are involved. For example, one member of the Harris County cohort was found to have two genetic variants that likely contributed to cause of death, suggesting multiple pathogenic pathways. While this individual had genetic variations in two cardiac genes, not necessarily all SIDS/SUD deaths are restricted to cardiac etiology. Alternate SD hypotheses include genetic variations leading to dysfunctional inflammatory, metabolic, central respiratory or neurological pathways; all of which could result in a negative anatomical autopsy (Van Norstrand and Ackerman 2010). In particular, significant evidence suggests many SIDS cases may be at least partly due to abnormalities in brainstem development. Genetic defects can alter critical period neuroplasticity including the expression and regulation of

multiple neurochemicals. Several studies investigating SIDS suggest disruption of the homeostasis of the serotonergic signaling pathway leads to dysfunctional sleep arousal and respiratory function (Broadbelt et al. 2011; Kinney et al. 2011; Machaalani and Waters 2014). However, some central nervous system disorders, such as epilepsy, are neurogenic but can manifest in lethal cardiac complications (Finsterer and Karim 2014).

Alternatively, SD may not always be explained by genetic defects, but could be due other aberrant signaling pathways or protein expression. Regardless, several genes implicated in sudden unexplained death of epilepsy (SUDEP), inflammatory, and metabolic pathways were included in our assay (Table 1). While none of the cohort with reportable genetic variants using the data filtration strategies presented here were positive for defects in these genes, it is important to consider multiple pathogenic molecular pathways and polygenic causes to SDs in the design of a more comprehensive and useful NGS molecular autopsy. Furthermore, the technology and strategies presented here could be applicable in death investigations involving other genetic diseases and anomalous molecular pathways.

Discovery and assessment of genetic variants related to sudden death and / or other diseases is a continually evolving field. As evidence of the dynamic nature of genetic diagnostics, next generation sequencing kits and software packages, such as Illumina Cardio Panel and VariantStudio software, for purposes of identifying variants in crucial cardiac genes have recently become more widely available to researchers at more accessible costs. In an effort to continue implementing postmortem genetic screening at the Harris County Institute of Forensic Sciences, samples from this study are being reevaluated with new technology, and additional putative genetic variants have already been detected. These new technologies and discoveries highlight the need for genetic testing to be considered in death investigations.

Conclusion

In conclusion, the present study represents the largest heterogeneous cohort of SD cases evaluated by a large targeted sequencing panel for genes associated with SIDS and SUD. This genetic screening strategy offers a cost-effective tool for medical examiners for diagnosis in autopsy negative cases of SD in the young. In addition to being diagnostic in nature, these screenings allow for affected families to make appropriate choices regarding medical testing, treatment, and lifestyle. The work presented here demonstrates the necessity to establish an interdisciplinary program for genetic result interpretation, disclosure, and follow-up counseling that accompanies the implementation of postmortem genetic screening. As the dynamic field of molecular diagnostics advances, molecular autopsy tools have begun to be within reach as a new standard of care for autopsy negative cases.

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VI. Dissemination of Research Findings

Journal articles

Methner DNR, Scherer S, Welch K, Walkiewicz M, Eng CM, Belmont JW, Powell MC, Wolf DA, Sanchez LA, Kahn R. 2016. Identification, verification and reporting of causal genetic variants in a large cohort of sudden infant death syndrome and young sudden unexplained death victims. *Manuscript submitted for publication.*

McGuire AL, Moore Q, Majumder M, Walkiewicz M, Eng C, Belmont J, Nassef S, Darilek S, Rutherford K, Pereira S, Scherer S, Sutton VR, Wolf D, Gibbs R, Kahn R, Sanchez L. on behalf of the Molecular Autopsy Consortium of Houston (MATCH). 2016. The ethics of conducting molecular autopsies in cases of sudden death in the young. *Genome Research* (in press).

Moore Q, Majumder MA, McGuire AL. Ethical and legal challenges associated with public molecular autopsies. 2016. *The Journal of Law, Medicine & Ethics* (in press).

Seminar presentations

Methner DN – "Identification, verification and reporting of causal genetic variants in a large cohort of sudden infant death syndrome and young sudden unexplained death victims." Green Mountain DNA Conference, July 2015, Burlington, VT.

Kahn R – "The Molecular Autopsy: Collaborative sequencing, verification, and reporting of causal mutations in a large cohort of infant and young adult sudden death victims." American Academy of Forensic Sciences Annual Meeting, February 2015, Orlando, FL.

Methner DN – "Next generation sequencing technology for the identification of genetic markers associated with sudden unexplained death and sudden infant death syndrome." Association of Forensic DNA Analysts and Administrators Summer Meeting. August 2014, Houston, TX.

Weymouth KS – "Next generation sequencing: a molecular genetics autopsy tool for identifying potential genetic causes for unexplained deaths in infants (SIDS) and children and adults (SUDs)" Green Mountain DNA Conference, July 2013, Burlington, VT.

Kahn R – "Cardiac molecular genetics for the medical examiner." National Institute of Justice Conference, June 2012, Arlington, VA

Poster presentations

Methner DN, Weymouth K, Scherer S, Powell M, Welch K, Kahn R– "Next generation sequencing technology for the Identification of genetic markers associated with sudden unexplained death and sudden infant death syndrome." International Symposium on Human Identification, September 2014, Phoenix, AZ.

Professional development

McGuire AL- "Ethics and Policy Project" Harris County Institute of Forensic Sciences Harris County Institute of Forensic Sciences – Medical Examiners Meeting (provided 1.5 hours of CME credit), October 2014, Houston, TX.

Nassef S and Darilek S- "Genetic Counseling: Facilitating the results disclosure process." Harris County Institute of Forensic Sciences – Medical Examiners Meeting (provided 1.5 hours of CME credit), October 2014, Houston, TX.