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The Value of Post-Mortem Genetic Testing in Individuals with Sudden Death in a Large Urban Setting: A Custom Cardiovascular Genetics Panel in an Internal Molecular Genetics Laboratory at a Medical Examiner's Office

The Value of Performing a Post-Mortem Cardiovascular Genetic Panel Test

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Abstract:

Purpose: To determine the utility of a post-mortem 95 cardiac gene panel in the diverse NYC population through examining the positive phenotypic predictors of clinically actionable gene variants as in those with sudden death.

Methods: 254 participants with sudden death underwent post-mortem testing through a 95 cardiac gene panel between Oct 2015-Feb 2018. NGS and variant interpretation was performed internally at the NYC Office of the Chief Medical Examiner (OCME) following ACMG guidelines. Medical information was collected from the OCME internal records. Chi-square tests were used to investigate categorical predictors of pathogenic genetic test results.

Results: Of 319 genetic test results, 51.4% (n = 164) were VUS, 9.1% (n = 29) were clinically actionable, and 39.5% (n = 126) were negative. Clinically actionable variants were found in 51 of the 95 genes sequenced. Positive predictors of pathogenic genetic test results were significant personal medical history, significant family history, and heart findings on autopsy.

Conclusion: The results support widespread testing on all sudden death cases, however, this may not be feasible everywhere due to limited resource or financial allocations. From this study we were able to determine inclusion criteria for post-mortem genetic testing for heritable cardiac conditions.

Key Words: post-mortem; genetic test; genetics; cardiac genes; sudden death

Introduction

In the state of New York, a medical examination in the form of an autopsy can be performed on any death which appears to be the result of an act of violence, unlawful, or suspicious circumstances, a death of any person confined in a public institution other than a hospital, infirmary, or nursing home, and any death such that no cause of death can be identified by a physician (N.Y. County Law § 673). The purpose of these autopsies is to determine the cause of death for legal and public health purposes, and for closure for the family. During this time, the medical examiner can perform any tests that are deemed necessary to determine the cause of death (CDC, 2014). The determination of sudden unexplained death (SUD) as the cause of death upon autopsy describes sudden, natural, unexpected deaths, which may have a cardiovascular or unknown etiology. A negative autopsy is declared when the death cannot be attributed to any pathological or chemical changes in any of the examined systems (Semsarian & Hamilton, 2012).

Sudden death is any death occurring within an hour of the onset of symptoms or (if death was unwitnessed) death after the individual was seen functioning normally within twenty-four hours of being declared deceased (WHO, 2004). The National Association of Medical Examiners (NAME) suggests that certain cases of sudden death be considered suspicious for a genetic etiology, such as drownings, single motor vehicle accidents, unexplained seizure in a young person, identified cardiomyopathy or aneurysm, sudden unexplained death in an individual with a family history of heart disease or SUD, and any deaths that are sudden and with no clear cause (Middleton et al., 2013). Included within the scope of testing available to the medical examiner are molecular genetic tests in which preserved tissue or blood samples are sequenced to determine if there are any variants in important genes which may be implicated in the sudden death.

Life-threatening arrhythmias caused by channelopathies and cardiomyopathies can lead to sudden death. Cardiac channelopathies cause disruptions in the typical flow of ions, leading to an irregular electrochemical gradient in the heart (Fernández-Falgueras, Sarquella-Brugada, Brugada, Brugada, & Campuzano, 2017). This may lead to abnormalities in the heartbeat, causing malignant arrhythmias in anatomically normal hearts. Therefore, after a standard autopsy, a channelopathy will not be identifiable without the utilization of molecular testing and could result in a negative autopsy. Cardiomyopathies are often associated with structural changes in the heart which interfere with the function of the cardiac muscles (Fernández-Falgueras et al., 2017). However, some of these structural variations are subtle or localized and may be missed upon physical examination. It is also possible that the defect is in a prodromal stage occurring before signs of myocardial dysfunction are visible. Both of these conditions can cause the heart to suddenly stop beating, cutting off the blood flow to the brain and other vital organs, resulting in death without swift intervention.

According to the National Society of Genetic Counselors, the goal of post-mortem genetic testing through molecular autopsy is to determine the cause of death and consequently identify if blood relatives are at risk (NSGC, 2017). Many heritable arrhythmic and structural syndromes are inherited in an autosomal dominant manner, meaning that if a decedent has a pathogenic variant, there is a 50% chance any of their first-degree relatives also has this variant (Bezzina, Lahrouchi, & Priori, 2015). This creates options for family members to be tested for the familial variant, which can lead to increased surveillance to those at a high risk of having a

cardiac episode. Furthermore, according to the American College of Medical Genetics and Genomics (ACMG), a definitive diagnosis through genetic testing provides the ability for families to receive early intervention, independent of whether the affected family member benefited directly from this diagnosis (Watson, 2015).

The research performed in the field of post-mortem genetic testing for cardiac genes has identified a range of results, with some consistencies in findings as well as some incongruities and limitations. Cardiac gene panels are usually targeted to specific loci likely to return results (Semsarian & Hamilton, 2012) and provide insight into undiagnosed disease as a contributor to the death. Studies have shown that 13% to 41% of the time a pathogenic or likely pathogenic genetic change can be found through post-mortem testing in sudden unexplained death (Campuzano et al., 2014; Christiansen et al., 2016; Lahrouchi et al., 2017; Stattin et al., 2016; Tester, Medeiros-Domingo, Will, Haglund, & Ackerman, 2012). These numbers are in general agreement with a National Association of Medical Examiners position statement suggesting that genetic testing can help to identify the cause of cardiac death in either the deceased or the family members as much as 40% of the time (Middleton et al., 2013). The ACMG asserts that the clinical utility of medically actionable diagnoses which inform causality, prognosis, and treatment are important to detect (Watson, 2015). Data reported by two different studies provided evidence that a clinical diagnosis was able to be established in family members of a decedent with a pathogenic variant in a cardiac gene approximately 12-13% of the time (Bagnall et al., 2016; Lahrouchi et al., 2017). In studies examining genotype-phenotype correlations for pathogenic variants, there is general agreement that adrenergic circumstances, including physical activity, intense emotion, or stress, are often correlated with positive genetic test results (Anastasakis et al., 2016; Christiansen et al., 2016; Lahrouchi et al., 2017; Stattin et al., 2016; Tester et al., 2012). The same association is seen with personal and family histories of cardiac events (Lahrouchi et al., 2017; Stattin et al., 2016; Tester et al., 2012). There is less agreement when it comes to the effects of age (Campuzano et al., 2014; Tester et al., 2012), sex (Stattin et al., 2016; Tester et al., 2012), and the comparative utility of testing for the different types of cardiac problems (Anastasakis et al., 2016; Campuzano et al., 2014; Christiansen et al., 2016). The available body of research is limited between races and ethnicities for the sudden cardiac death population, and often the mechanism of defining race is poorly defined or absent, further obscuring the data. One study claimed that the incidence of SCD is significantly high amongst all

races, but accounts for overall deaths in 63.7% of Caucasians, 62% of African Americans, 60.5% of Native Americans, and 55.2% of Asians (Zheng, Croft, Giles, & Mensah, 2001). Additionally, Hispanics appeared to exhibit a lower proportion of cardiac deaths than non-Hispanics (Zheng et al, 2001). The limited research on SCD risk associated with ethnicity highlights the necessity for further study, especially as it relates to the diversity in New York City. These discrepancies may exist because of the different ethnic diversity of populations being represented in each of these studies; however, they may also exist because of some limitations in the literature as a whole.

There are multiple examples of limitations present in research on sudden cardiac death to date. One such example is represented by the differences in classification of a negative autopsy both between studies and within studies when autopsies were performed at multiple locations. Additionally, for research performed prior to 2015, there was not yet standardization for variant interpretation. These guidelines were released by The American College of Medical Genetics (ACMG) in 2015 (Richards et al., 2015). Further limitations included the number and type of genes being tested differing between studies, and the focus of much of this research on variably defined age groups.

Individuals at risk for SCD are difficult to identify because often the first sign of cardiac disease is sudden death, with no other symptoms or warning signs (Mozaffarian et al., 2016). Moreover, due to the lack of autopsy findings after death, it can be difficult to diagnose these cases, leaving many deaths unexplained. Therefore, it is of great benefit to recognize those who carry a pathogenic gene variant in order to be able to enact preventative measures. To date, many genetic variants have been identified that predispose an individual to SCD. Performing post-mortem genetic testing on victims of SCD suspected to have a pathogenic variant not only has implications for the family, but this type of testing can have broader impacts on public health policies and healthcare as a whole. The characterization of these deaths can help inform protocols, both for the management of other at-risk patients with these conditions and for optimizing the approach to genetic testing of heritable cardiac conditions.

The current research surrounding sudden cardiac death is substantial but has the potential for massive improvements. The goal of the present study performed in conjunction with the Office of the Chief Medical Examiner (OCME) in New York City was to improve on this research by studying the utility of a post-mortem 95-gene cardiac panel on individuals falling into the SCD classification. Currently, the NYC OCME is the only medical examiner's office in

the country which performs in house genetic testing on autopsy samples; therefore, it is particularly important to understand the effectiveness and utility of this particular panel when examining the New York population. It is understood that NGS is useful for studying diseases with large heterogeneity, such as cardiac disease, as long as a stringent filtration process is used when approaching that the large variant dataset that accompanies with large panels (Farrugia, Keyser, Hollard, Raul, Muller, & Ludes, 2015; Lin, Williams, Wang, Coetzee, Zhou, Eng, Sampson, & Tang, 2017). The OCME developed a large custom NGS panel. As cardiac conditions are known to be very heterogeneous, both in disease expression and in genetic origins, the large panel was developed to identify the underlying genetic cause in many cases, while still providing results associated with clinical actionability when pathogenic variants were found. This study looks at all cases of sudden death, regardless of autopsy conclusion and age of decedent. The 95-gene panel was used consistently for analysis of all cases and variant interpretation was done by OCME genetics staff following ACMG guidelines for variant interpretation (Richards et al., 2015; Yin, 2017). Through this analysis, we aim to describe the genotype and phenotype of decedents with pathogenic changes to determine a profile of the genetic SCD case, validate the specific gene panel in the New York population, and identify how family members can influence and be influenced by testing.

Methods
Data Collection and Participants

Between October 2015 to February 2018, 267 deceased individuals whose deaths can be classified as "sudden death" underwent gene testing through a customized 95 cardiac gene panel at the New York City Office of the Chief Medical Examiner. Further data was collected on these individuals from the OCME internal records including scene investigation by police and family interview by a certified physician assistant, complete gross autopsy, neuropathologic and cardiac pathology examinations, toxicological records, and microbiological tests and metabolic screens in infants. Further information was also obtained from genetic reports and medical or hospital records where available. Data collected for each case includes age, ethnicity, sex, genetic test results, anatomical and heart findings, toxicology, circumstances of death (i.e. presenting symptoms, activity at death, etc.), personal medical history, family medical history, and genetic counseling services received (**Table 1. Summary of Significant Personal and Family Histories Collected**). All demographics were obtained from internal OCME records, in which

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race and ethnicity were grouped together. As race is a social construct, henceforth we will refer to these demographics collectively as ethnicity.

Of the study population, 13 cases were excluded from further analysis beyond demographics, as autopsy information was unavailable due to objection by next of kin, autopsy having been performed at another medical examiner's office, or inaccessible autopsy report at the time of data collection.

This study is not regulated by 45 CFR Part 46, Human Subjects Protection, because post-mortem samples are not considered human subject and only cadaver specimens were used. However, this research was approved by the Chief Medical Examiner and by the general counsel at the OCME of NYC and deemed exempt from Institutional Review Board approval by a committee at Sarah Lawrence College.

Genes

The panel analyzes 95 disease genes associated with cardiac channelopathies such as Long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia, as well as cardiomyopathies including hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, and left ventricular noncompaction cardiomyopathy. Most of the disorders on the panel are inherited in an autosomal dominant fashion, but a few are inherited through an autosomal recessive pattern or an X-linked pattern. A full list of genes and associated disorders is included (**Figure 1. Results of the Next Generation Sequencing Panel for Variants)** (Tang, 2016).

Testing Methodology

The test was developed and performed in the Molecular Genetics Laboratory at the City of New York Office of the Chief Medical Examiner and done through analysis of genomic DNA from dry bloodstain cards or post-mortem tissue samples collected at the time of autopsy and preserved in RNAlater. The institutional laboratory is accredited by The College of American Pathologists (CAP), but the specific test has not been cleared by the FDA. The analysis performed includes sequencing of both coding regions and splice sites of all 95 genes. Oligonucleotide-based in-solution target capture (Haloplex Target Enrichment System, Agilent Technologies) was performed, followed by next generation sequencing. The genomic reference coordinate used is GRCh37/hg19. Illumina Miseq was used to perform primary sequencing data analysis to generate a sequencing read. Secondary sequencing was then performed using

NextGENe (SOFTGENETICS) and included the delivery of alignment data, variant identification, and filtering the sequence by quality. Thirdly, variant classification was performed by Geneticist Assistant (SOFTGENETICS) which accepts raw sequencing data and synthesizes information from the following sources:

Functional Prediction information: SIFT, PolyPhen2, LRT, Mutation Taster, FATHMM, CADD & Mutation Assessor

Disease association: ClinVar, OMIM & COSMIC

Conservation scores: PhyloP, GERP++, phastCons & SiPhy

Population frequencies: 1000 Genomes and Exome Variant Server

Further sequence analysis was performed to ensure quality, and when possible, follow up confirmation with Sanger sequencing in regions with no coverage, low coverage (<30X), and regions determined to have clinically significant alterations. The test detects >98% of substitution variants (95% CI [98%, 99%]) (Tang, 2016).

Variant Interpretation

Evaluation of clinically significant variants was performed in accordance to guidelines put forth by the American College of Medical Genetics and Genome (ACMG) and the Association for Medical Pathology (Richards et al., 2015). The New York City Office of the Medical Examiner is home to a molecular genetics laboratory, so all interpretation is done internally. A bioinformatic pipeline, The Geneticist Assistant, is used to perform initial variant interpretation from the raw data. Following this step, review of the data and further interpretation and classification of reportable variants (pathogenic, likely pathogenic, variants of uncertain significance) are done manually by the director of the laboratory and the genetic counselor. The first step in classifying reportable variants is to determine if a sequence variant has been previously reported. To do so, the variants are searched in disease databases such as the Human Gene Mutation Database, ClinVar, and PubMed. The purpose of this is to determine if the variant has been shown to have clinical relevance, if there is data from family or cohort studies, or evidence of the deleterious or benign functionality of the variant. If the variant has never been reported in the databases or in the literature, the evaluation of the variant consists of several factors which contribute towards its classification. It is essential to assess the pattern of inheritance, the type of variant (such as: loss of function, missense, in-frame insertions or deletions), and its location in the protein or splice site of the gene. Additionally, databases such

as Exome Aggregation Consortium (ExAC), 1000 genome, NHLBI Exome Sequencing Project (ESP), The Genome Aggregation Database (gnomAD) are used to ascertain the minor allele frequency of the variant in the general population, while computational tools such as Polyphen2, Provean, or MutationTaster are utilized to determine the effect the variant may have on protein. Finally, all relevant autopsy findings, medical history, and family history will be included to determine the variant classification. All of the information gathered during this analysis will provide a sense of how the variant may affect the individual (Tang, 2016).

Reporting

After all variants have been evaluated, each single variant is classified into one of five categories which include: pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign. All pathogenic, likely pathogenic, and uncertain results are reported because they are considered to be clinically significant and will be referred to as "reportable results," benign or likely benign variants are not reported. In this study, all pathogenic and likely pathogenic variants were combined for analysis and will be referred to as "clinically actionable variants". These classifications were merged because they both routinely warrant recommendations for clinical management and cascade genetic testing based on the variant finding, as opposed to other variants such as negative or uncertain results, which are not necessarily associated with an impact to clinical management or with recommendations to follow up with clinical genetic services. Variant nomenclature follows guidelines provided by the Human Genome Variant Society (HGVS). As the field of genetics and the study of new genes and mechanisms are being updated all the time, all variant interpretations are subject to modification and reclassification at any time. Any first-degree blood relatives of a decedent who is found to carry a clinically significant variant may be recommended to receive clinical evaluation and genetic counseling (Tang, 2016).

Test Benefits and Limitations

The benefit of this panel lies in the fact that it contains a large number of well understood disease genes with associations to treatable conditions and uses Next Generation Sequencing (NGS). NGS has proven to be a sensitive and specific valuable tool in post-mortem genetic testing because of its high throughput and low cost, which also results in a stringent variant filtration approach (Chanavat, Janin, & Millat, 2016; Farrugia et al, 2015). Also, using the Haloplex Target Enrichment System, there is a low (2%) false positive rate due to technical

limitations. However, analysis through NGS is also known to result in the accumulation of rare, unknown variants (Farrugia et al, 2015). The limitations of performing this type of sequencing include the exclusion of non-coding material, and the inability to detect inversions and deletions or large copy number variants. Detection is also low in highly homologous or repeat regions. Therefore, there could be a genetic alteration in one of the 95 genes that is outside of the region that was tested on the panel but could still impact health and have implications in cause of death. There could also be a genetic alteration in a gene that was not studied on the panel but plays a role in cause of death. Other comparable commercial or clinical panels used for heritable cardiac diseases may include different genes or testing methodologies. Finally, any post-mortem changes to the quality of the blood sample collected during autopsy could impact results (Tang, 2016).

Statistical Analyses

The data was downloaded from Excel into SPSS version 24 for statistical analysis. Chi-square tests were used to investigate categorical predictors of a pathogenic or likely pathogenic variant. P levels of $p < 0.05$ was used to indicate statistical significance.

Results

The total sample (n = 254) was 64.2% male (n = 163) and 35.8% female (n = 91), and 40.8% Black (n = 102), 31.6% Hispanic (n = 79), 22.4% White (n = 56), and 5.2% Asian/Pacific Islander (n = 13). The majority of cases consisted of those falling into the adult classification (52.7%; n = 134). This was followed by the infant category (25.6%; n = 65), then young adults (11.4%; n = 29), and finally, children (10.2%; n = 26). The mean age of the sample was 24 years, while the median was 26.5 years. There was a standard deviation of 18.5 years. Of the total sample (n=254), 22 individuals (9.4%) were exercising or exerting energy at the time of death, 129 individuals (55.4%) were at rest or sleeping at the time of death, 82 (35.2%) were engaged in an activity other than rest/sleep or exercise/energy exertion at the time of death, and for 24 of the individuals, activity at death is unknown (**Table 2. Demographics and Clinically Actionable Findings**).

Genetic testing was performed and rendered 319 results in 51 of the 95 genes analyzed (**Figure 1. Results of the Next Generation Sequencing Panel for Variants**). 60.5% (n=193) of all results were reportable (pathogenic, likely pathogenic, variant of uncertain significance). In 39.5% (n = 126), no reportable variants were identified. Variants of uncertain significance (VUS)

were found in the highest proportion of the sample (51.4%; n = 164). 9.1% of the sample (n=29) had clinically actionable variants, 12 of the 29 were female and the remaining 17 were male. A total of 48 subjects were reported to have greater than one variant detected by testing: 37 people had 2 variants identified, 6 people had 3 variants identified, 4 people had 4 variants identified, 1 person had 5 variants identified. No individual was found to have more than one pathogenic or likely pathogenic variant; however, 12 of the 29 cases with clinically actionable variants (41.4%) were found to have one pathogenic/likely pathogenic variant in addition to at least one VUS. 58 of the 193 (30.1%) total reportable variants were novel, meaning that they had not previously been identified in the population. Of these, 12 out of 58 (20.1%) were clinically actionable novel variants.

Through Chi-square analysis, positive predictors of having a reportable genetic test result were identified. Having a significant personal medical history was a positive predictor for identifying a likely pathogenic or pathogenic variant on the 95-gene panel ($\chi2 = 13.82$, df = 2, p < 0.01). Cases with a pathogenic or likely pathogenic finding had significant personal histories (17.4% versus 4.4%), while those without significant personal histories were more likely to have VUS (54.4% versus 46.7%) and more likely to receive a negative result (41.4% versus 35.9 %) **(Figure 2. Significant personal medical history in those with clinically actionable results)**. In addition, positive predictors of discovering a reportable variant included the presence of heart findings ($\chi2 = 12.75$, df = 2, p <0.01). More of those individuals with heart findings were found to have clinically actionable variants (12.1% versus 3.1%), or VUS (53.4% versus 43.8%). There were also more individuals with heart findings who had pathogenic or likely pathogenic results (12.1% versus 3.1%) and more of those without heart findings received negative test results (53.1% versus 34.4) **(Figure 3. Heart findings found in those with genetic test results)**. A significant family history ($\chi2 = 12.72$, df = 2, p <0.01) was also determined to be associated with reportable results. More individuals with a significant family history had VUS (54.1% versus 37.5%) or pathogenic/likely pathogenic (21.3% versus 6.3%) results found on genetic testing, whereas more individuals (56.3%) without a significant family history were negative than those with a significant family history (24.6 %).

When examining age distribution, there were significant associations based on age groups ($\chi2 = 18.94$, df = 6, p<0.01). Those in the 1 to 18 years of age category had the highest percentage (63.9%) of variants of uncertain significance. Those under a year old had the highest

percentage of negative results (53.9%), while those from 1 to 18 years had only 27.8% as negative. Significant associations were also found between genetic test results and ethnicity ($\chi 2$ = 15.68, df = 6, p<0.05). VUS results were identified more frequently in individuals with Asian/Pacific Island ancestry (76.2%) than for Blacks (59.4%), Hispanics (46.5%), Caucasians (36.9%). Genetic test reports with negative results were more frequent in Caucasians (52.3%), Hispanics (43.6%), and Blacks (32.8%) than for Asian/Pacific Islanders (14.3%). There were no significant differences in the genetic test results based on sex ($\chi 2$ = 2.50, df =2, p = 0.29). Males and females shared similar proportions of result classifications (**Table 2. Demographics and Clinically Actionable Findings**).

There were no statistically significant differences in the results by type of activity at death ($\chi 2$ = 2.96, df = 2, p= 0.23). Slightly more of those individuals exercising or exerting energy (57.7%) or resting/sleeping (46.7%) had variants of uncertain significance. Additionally, those individuals who received negative results were slightly more likely to have died while resting or sleeping (47.7%) versus those were exercising or exerting energy at the time of death (30.8%).

An examination of the genetic counseling encounters revealed that there were recommendations in 116 cases for families to receive genetic counseling follow up. Of these, only 25 families were known to have received genetic counseling. All cases who received clinically actionable genetic test results (n = 29) were recommended for next of kin to seek genetic counseling. In this subset, only 34.5% (n = 10) families were known to receive genetic counseling. All 10 of these families were found to have significant family histories during the course of the genetic counseling session. Of the remaining families, 18 did not have genetic counseling, and it is unknown as to whether the last remaining family had genetic counseling. Only 3 of the 18 families who did not have genetic counseling were reported to have significant family histories by investigational personnel. 13 of the 18 families had unknown family histories, and the remaining 2 families were reported to not have significant family histories.

Discussion

Implications

The present study examined the testing yield of post-mortem genetic testing in a sudden death population that is representative of typical casework. Contrary to our heterogeneous

sample, many previous studies have examined only sudden unexplained (or autopsy negative) cases or cases with specific heart findings (i.e. cardiomyopathy) in their analysis. In this study, 29 clinically actionable results were detected in 29 of 254 individuals (11.4%) examined. The findings resulted in a lower yield than found in previous research which reported yields from 13% up to as high as 40% (Campuzano et al., 2014; Christiansen et al., 2016; Lahrouchi et al., 2017; Stattin et al., 2016; Tester et al., 2012). In other studies, the yield may have been higher due to sample biases in selecting cases with specific heart findings or cases where no cause of death could be determined. We attribute our lower yield to our heterogeneous sample. Using this method, removing bias from our sample, there is inclusion of an increasing number of cases where cardio-genetics may not have been a factor in death.

However, despite this lower yield, our results indicate that there is value in testing all sudden death cases. In 11 cases out of the 29 (37.9%), there was an identified cause of death determined irrespective of genetic results, which means that these cases would have been overlooked in previous studies. This would mean that the at-risk family members eligible for testing would also have been missed in protocols including only sudden unexplained deaths. This information is useful because knowledge of one's genetic results and therefore risk of cardiac related deaths may inform lifestyle behavior or play a role in more complex diseases. Additionally, these results bring to light the subjectivity in cause of death determination between medical examiners and between institutions. This study highlights the benefit of doing post-mortem genetic testing in individuals who have had sudden death, even for those in which the cause of death may not, on autopsy, appear to be related to the cardiac system, such as cases of acute drug overdose.

In addition to identifying heritable risks, this study identified 58 novel variants, which broadens understanding of human variation and also identifies points of further research in variant interpretation, as 46 of the 58 variants were classified as VUS. The findings of this study further contribute to the growing body of literature as nearly half (n = 12; 41.4%) of the 29 clinically actionable cases were previously absent from the literature and OCME's internal database. The large proportion of VUS (51.4%; n = 164) in the total sample (n=254) is most likely accounted for by the nature of NGS, revealing a large preponderance of rare missense variants, as well as the generally large and heterogeneous characteristics of genes that influence cardiac conditions.

Of the 95 genes analyzed as a part of this study, we found variants in 51 (53.7%). We theorize this is because of the breadth of genes we opted to include in our panel. In addition to choosing major genes for conditions (such as *KCNQ1*), we also analyzed well understood genes in which pathogenic variants are a rare cause of the cardiac conditions (such as *NPPA*). The rationale of choosing such a large panel is that it is in the decedent's and their surviving family's best interest to test widely - including rare cardiac disease genes - to minimize overlooking a heritable explanation for the sudden death. This is possible at the OCME, because the resources and workforce are available to sequence, interpret, and make recommendations based on variants found in any of the genes assessed, regardless of whether it is a major or minor disease-causing gene. However, due to the low sample size of 254, we would not expect to have a high frequency of variants in rare genes, explaining why in 46.3% (n = 44) of genes sequenced, no reportable variants were discovered.

There were no significant differences found between groups when looking at sex, which was surprising considering the much higher ratio of males to females in the total cohort. However, the number of males to female in the clinically actionable result category was more equivalent. In previous research, there have been variable conclusions regarding the effect of sex on genetic test results. We theorize that due to the low yield of clinically actionable results in this study, we did not have enough individuals in this group to identify trends in sex, if any do exist.

In other demographic categories, significant differences were observed between groups. Though those who were reported as Asian/Pacific Islander made up a very small portion of the cohort, at only 5.1%, these individuals had a significantly higher rate of variants of uncertain significance, and lower rates of negative results than those of any other reported ethnicities (Black, Hispanic, White). We attribute this level of uncertainty to the lack of robust population databases for ethnicity matched controls. New York City is comprised of a diverse population, and the need for broader studies on varying ethnic backgrounds is blatantly apparent.

There was a significantly higher number of VUS found in the child classification than in any other age category. Classification of each variant was performed using ACMG guidelines, which includes the phenotype of the patient as supporting evidence for pathogenicity. Young individuals have the potential to be in a pre-symptomatic or prodromal stage, in which there is a reduced chance for disease signs and symptoms (i.e. significant personal history, such as fainting spells, or physical manifestations such as cardiomyopathy) prior to death. This reduces the

amount of phenotypic information available during variant interpretation. This lack of potential data supporting pathogenicity could have resulted in a higher number of VUS. The infant age group represented the highest proportion of negative results, which may suggest other mechanisms in infant death, including other heritable conditions not tested for on this cardiac gene panel. In the future, it may be valuable to examine this subset of the sudden death population with other gene panels for common infant onset conditions (such as seizure disorders) to better characterize hereditary in deaths in individuals under one year of age.

The type of activity at death was not found to be a significant predictor of having a reportable or clinically actionable result. This is inconsistent with previous literature, which found correlations between adrenergic circumstances at time of death and pathogenic genetic test results (Anastasakis et al., 2016; Christiansen et al., 2016; Lahrouchi et al., 2017; Stattin et al., 2016; Tester et al., 2012). As previously discussed, some of these studies may have had sample biases in selecting for those with certain findings or lack of findings on autopsy. The selected findings could be correlated with genetic diseases that are known to be associated with sudden death upon adrenergic exertion. The majority of those who were resting or sleeping at time of death received a negative or VUS result. We propose that this could be because most individuals under the age of 1, who received only negative or VUS results spend the majority of their day sleeping or at rest and may comprise a large portion of this group. Another explanation for the lack of significance in activity at time of death could be related to cases in which the death was not observed. It is possible that when the death was not observed that the activity at time of death could have been mislabeled as at rest.

The discovery of heart findings on autopsy was a positive predictor for having a pathogenic or likely pathogenic genetic test result. It is reasonable to believe that this is due to the effect that harmful variants in cardiac genes have on the cardiac system, resulting in signs of disease. This group also exhibited more VUS as well. In the absence of other types of evidence during variant interpretation, symptomology and phenotype are supportive of pathogenicity. Therefore, it would make sense that these variants would not be classified as fully negative, and also may not contain enough evidence to be labeled as pathogenic. Lack of heart findings on autopsy was a positive predictor for a negative genetic test result. These cases could represent a subset of the population who died for reasons other than cardiac problems.

More of those who had a significant personal history were found to have pathogenic or likely pathogenic findings, which is consistent with showing clinical symptoms of cardio-genetic disorders. This supports the value of increasing awareness that referrals to genetics are necessary in the presence of signs and symptoms that may be related to heritable cardiac conditions. Variants were also more likely to be reported in individuals who had a family history remarkable for cardiac-related incidents. Many cardio-genetic disorders are heritable, which is supported by this evidence for family history of cardiac conditions. This also emphasizes the importance of recognizing potential cardiac conditions present in families, as screening and treatment can be effective in reducing mortality. Furthermore, many cardiovascular genetic diseases exhibit variable expressivity and reduced penetrance, therefore it may be difficult to determine clinically who is at risk without the assistance of molecular confirmation.

This study supported the value of genetic counseling sessions as a part of post-mortem investigation in the presence of clinically actionable variants. This was demonstrated by the fact that every case with clinically actionable findings that underwent genetic counseling was found to have significant family histories (100%; n = 10). The cases that did not undergo genetic counseling (n = 18) were only found to have significant family histories in 3 instances (16.7%). The majority of the cases that did not receive genetic counseling were reported to have unknown family histories, which reflects the absence of a fully comprehensive medical review (**Figure 4. Significant family medical history in those with reportable results)**. Collecting an accurate family history is important for determining at-risk family members and elucidating segregation in the family. Furthermore, we postulate that following molecular autopsy, there is psychosocial benefit to receiving genetic counseling; however, in this study, not enough cases were seen by genetic counselors to support this, and further study should be pursued.

Limitations

A primary limitation of this study was that it relied heavily on investigation and police story reports to collect personal and historical narratives about the decedent. In these reports, race and ethnicity were used interchangeably, which potentially confounds results on correlations between genetic test results and ethnic backgrounds. It is unclear whether the results that we obtained were meaningful based on ethnicity or whether they were a further perpetuation of race as a social construct. Furthermore, investigation and police reports are variably comprehensive regarding details such as personal medical history and family medical history. Genetic

counselors are specially trained to collect personal and family medical history information. From the results, it is clear that the genetic counseling encounters rendered more details in family medical history. It is possible that in the investigation reports, pertinent information was missed. Part of this lack in genetic counseling encounters is due to the fact that the NYC OCME did not have a genetic counselor seeing families until September 7, 2016, as well as difficulties in next of kin following up after the genetic results of the decedent are discovered. It is possible that if more personal and family history was collected, different significant trends would have emerged. As discussed previously, information on phenotype and medical history is integrated into variant interpretation. Additionally, cascade testing in families contributes to interpretation, as through tracking co-segregation of symptoms (or absences of symptoms) in relatives with variants there can be a better understanding of whether a particular variant has deleterious effects. Therefore, evidence supporting pathogenicity rather than supporting uncertainty, could have been missed. Moreover, investigation and police story reports were also relied on for activity at time of death, and as discussed previously, relying on next of kin reports in unobserved deaths may lead to uncertainty in the results. Finally, due to the low number of genetic counseling encounters observed, we were unable to perform meaningful statistics or study the clinical utility of actionable results on family members.

Another limitation that exists is the examination of the relationship between heart findings and genetic test results. Heart findings were defined as any change or abnormality of the cardiac system found on autopsy. Therefore, in this study, individuals were labeled as having heart findings if they had changes in the cardiac system relating to any cause, including environmental. This limits the utility of the results found between heart findings and clinically actionable test results, as some heart findings being examined are due to external or environmental factors (i.e. necrosis of the cardiac muscle) or may be due to genetic conditions not being examined on the 95-gene panel used in this study (i.e. congenital heart defects). The purpose of examining heart findings is to determine if changes in the cardiac system are significant predictors of clinically actionable genetic test results. Through being broad in the definition of heart findings in this research, it is possible that a significant interaction was missed. Future research would benefit from differentiating heart findings based on whether they may be genetically derived (for example, cardiomyopathies detected on autopsy) or not, in order

to assess true predictive value of heart findings on genetic testing as a part of post-mortem analysis.

Conclusion

From these results, we are able to begin characterizing which individuals are most likely to return a clinically actionable variant on a cardiac gene panel after a sudden death. Due to limited resources available at many Medical Examiner's offices, widespread testing on all sudden death cases may not be feasible everywhere. However, this research supports the importance of testing all cases, as 11 out of 29 at-risk families would have been overlooked if their deceased relative was not tested due to having a cause of death after standard (non-molecular) autopsy. Though at the NYC OCME there is an in-house testing laboratory where extensive genetic sequencing and interpretation can take place, limitations in resource and financial allocation at other institutions suggest that guidelines defining which decedents to test to achieve the highest yield of clinically actionable variants may be necessary. Though more research is required in a larger population to establish formal guidelines, this study strongly supports the inclusion of remarkable family and personal medical history in providing a molecular genetic test post-mortem.

Furthermore, this study provides support for the utility of genetic counseling involvement in the process of post-mortem genetic testing, as it can elucidate medical histories more clearly and also target psychosocial concerns, though this was not able to be addressed in the present study. Future research on those who were able to receive genetic counseling should be conducted, ideally once this subset represents a higher proportion of the sample. Perhaps identifying the best time frame or method of communication for follow up with next of kin after identification of clinically actionable results would assist in culminating a higher number of genetic counseling visits.

Overall, due to the large yield of variants of uncertain significance, this study points out that there is a need for more research and data collection on variants discovered through Next Generation Sequencing in different populations. As more information is collected on variants in healthy populations and affected individuals, variants of uncertain significance can be further classified, which would be beneficial for underrepresented groups.

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Tables

**Table 1. Summary of Significant Personal and Family Histories Collected**

Significant Personal History	Significant Family History
Atrial fibrillation	Confirmed cardio-genetic diagnosis
Abnormal Imaging	Cardiac Disease
Bradycardia	Seizures
Tachycardia	Hypertension
Cardiomyopathy Diagnosis	Sudden Cardiac Death
Channelopathy Diagnosis	Young/Accidental Death
Chest Pain	Channelopathy Diagnosis
Syncope/Fainting/Dizziness	Congenital Heart Defect
Heart Disease	Chest Pain
Heart murmur	Myocardial Infarction
Hypertension	
Irregular Heartbeat	
Mitral Valve Prolapse	
Seizure	
Shortness of Breath	

Table 2. Demographics and Clinically Actionable Findings

| Demographics of Sudden Death Sample N = 254	N	%	Actionable Variants
Sex			
Male	163	64.2%	17
Female	91	40.8%	12
Age			
Infant	65	25.6%	0
Child	26	10.2%	3
Young Adult	29	11.4%	3
Adult	134	52.7%	23
Ethnicity			
Black	102	40.8%	10
Hispanic	79	31.6%	10
White	56	22.4%	7
Asian/Pacific Islander	13	5.2%	2
Activity at Time of Death			
Rest/Sleep	128	50.4%	9
Exercise/Energy Exertion	21	8.3%	3
Other	81	31.9%	13
Unreported	24	9.4%	4

Figures

**Figure 1. Results of the Next Generation Sequencing Panel for Variants**
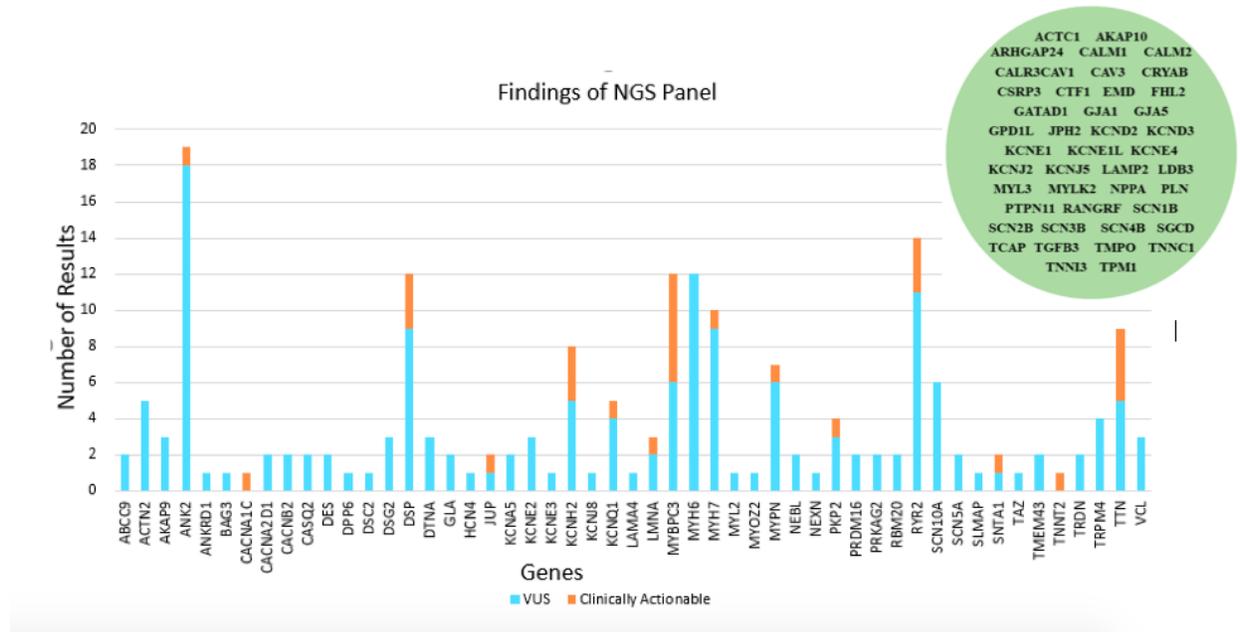
The number of VUS and clinically actionable findings in 51 genes on the panel are shown in the bar graph. The 44 genes on the panel which had no results are listed above in the green circle.

Figure 2. Significant personal medical history in those with clinically actionable findings


Significant Personal History in Those with Clinically Actionable Results
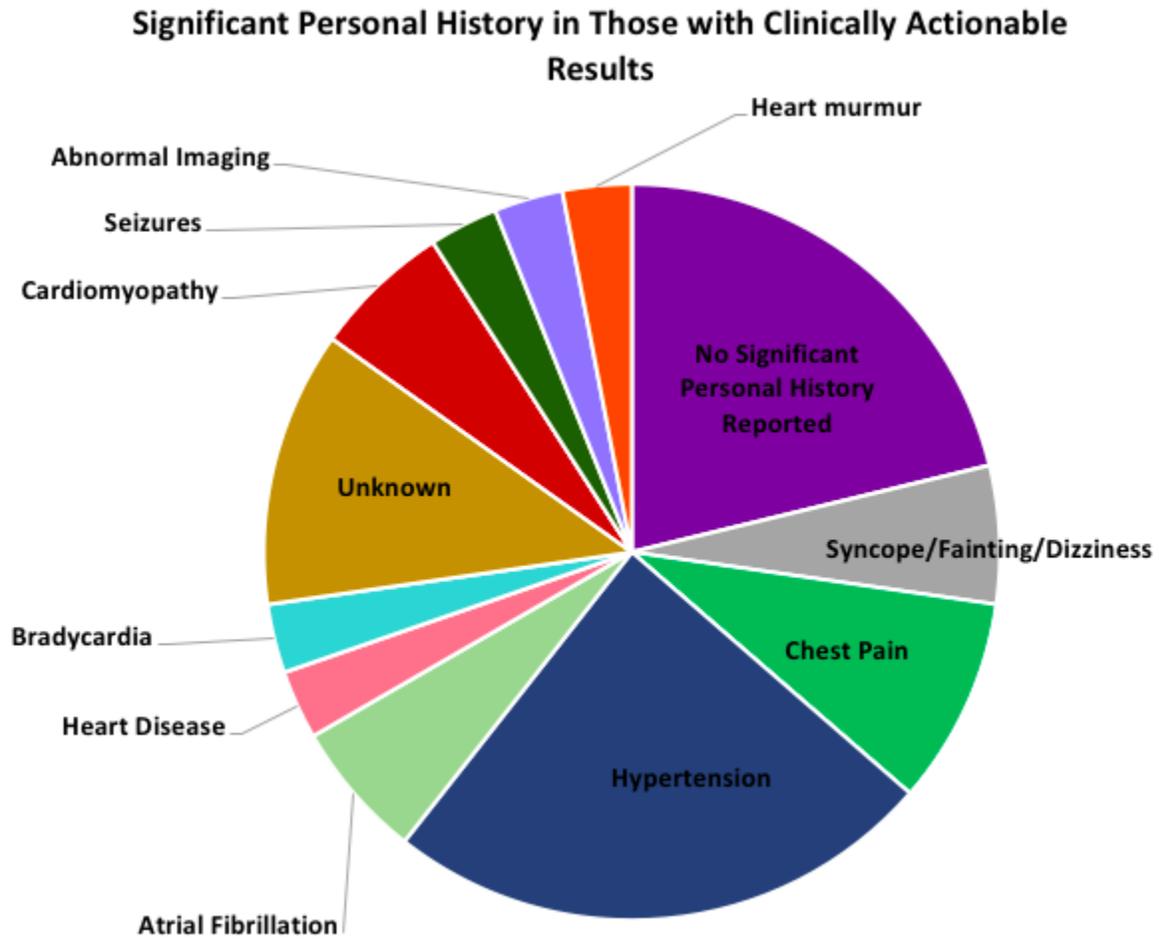
Significant personal history documented in those who had clinically actionable results.

Figure 3. Heart findings based on genetic test findings
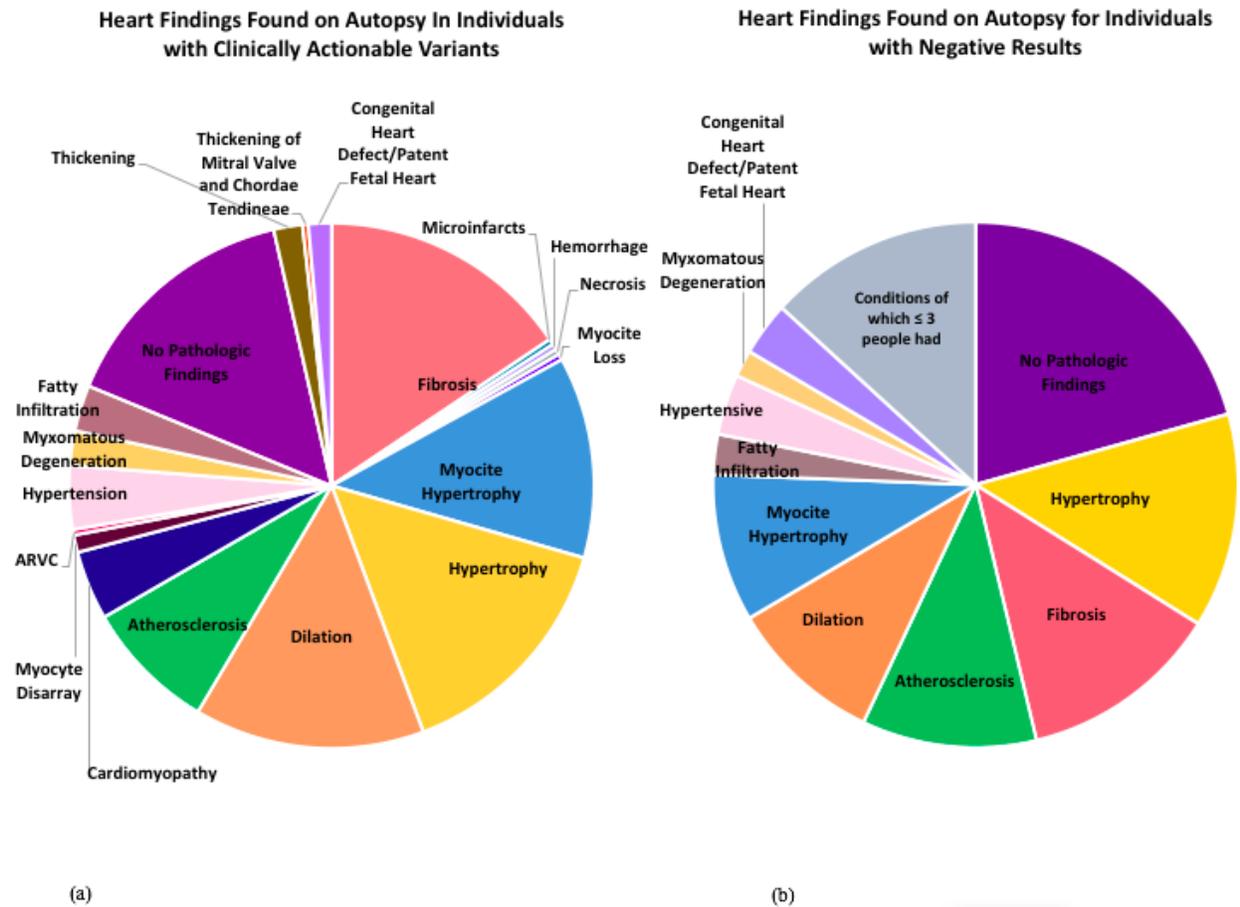
Prevalence of specific heart findings based on genetic test findings. Cases were classified for inclusion by highest degree of pathogenicity. (a) Heart findings in cases with reportable variants identified on NGS panel. Findings from cases with VUS findings were excluded if less than or equal to 3 individuals had that particular finding. Four cases were excluded due to lack of comprehensive cardiac examination due to organ donation. (b) Heart findings in cases with no reportable variants found on NGS panel.

Figure 4. Significant family medical history in those with reportable results
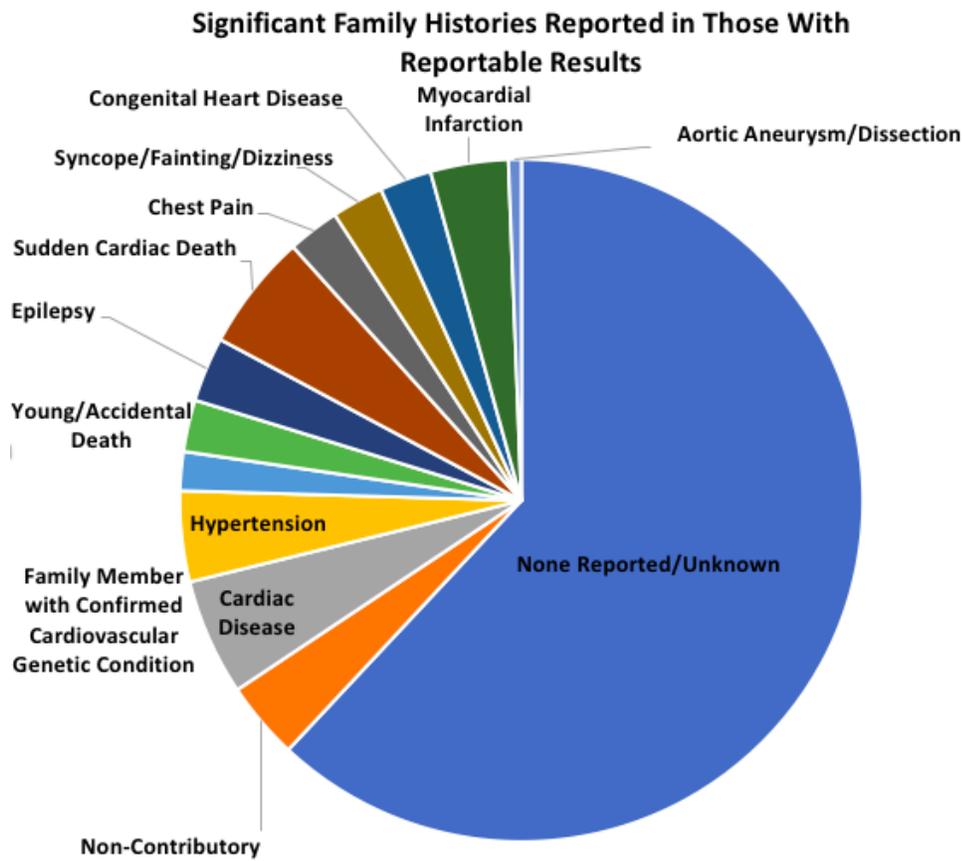
Significant family history documented in those who had clinically actionable results. Note the high frequency of unknowns in this category, theorized to be related to the low number of genetic counseling appointments.