Sudden unexpected death in a previously healthy individual is always a tragedy.

The investigation of such deaths involves the most comprehensive protocols: first, the Office of Chief Medical Examiner (OCME) sends a medical legal investigator to the scene to evaluate the circumstances of the death. Then, unless there is a religious rejection, a thorough and complete autopsy is performed by a medical examiner. OCME has specialized cardiac pathologists and neuropathologists who closely examine these important organs. Samples are collected at autopsy for toxicology, microbiology, genetic and metabolic tests.

When all the examinations are negative, we then perform genetic testing for cardiac diseases that may have no or minimal findings at autopsy.

The etiology of cardiac arrhythmia can be highly complex and heterogeneous. Cardiac arrhythmias can be caused by the diseases of the heart, such as the conduction system disorders and cardiac muscle system disorders. They can also be caused by disorders outside the heart, such as epilepsy or brainstem dysfunction, kidney or endocrine system disorders that disturb the ion homeostasis, metabolic diseases that affect energy production or immune system disorders.

OCME is currently focusing on the heart diseases. Because conduction system disorders do not change the morphology of the heart and the heart muscle change in children can be subtle, molecular testing the disease genes becomes essential.

In our lab, we developed a 95 cardiac disease gene panel. It includes 45 genes for conduction system disorders, such as long QT syndrome and Brugada syndrome that are commonly known as channelopathies. It also includes 50 genes responsible for various types of cardiomyopathies, such as the hypertrophic cardiomyopathy, dilated cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy.
## 95 Cardiac Disease Genes

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Gene Names</th>
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<tr>
<td>Conduction System Disorders (LQT, Brugada, SQT, AF, VF)</td>
<td>ABC9, AKAP10, AKAP9, ANK2, ARHGAP24, ACNA1C, CACNA2D1, CACNB2, CALM1, CALM2, CASQ2, CAV1, CAV3, DPP6, GJA1, GJA5, GP1D1, HCN4, KCNA5, KCND2, KCND3, KCNE1, KCNE2, KCNE3, KCNE4, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, NPPA, PRKAG2, RA, NGRF, SCN10A, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SLMAP, SNTA1, TRDN, TRPM4</td>
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<tr>
<td>Cardiomyopathy (HCM, DCM, LVNC, ARVD)</td>
<td>ACTC1, ACTN2, ANKRD1, BAG3, CALR3, CRYAB, B, CSRP3, CTF1, DES, DSC2, DSG2, DSP, DTNA, EMD, FHL2, GATA1, GLA, JPH2, JUP, LAMA4, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPN, NEBL, NEXN, PKP2, PLN, PRDM16, PTPN11, RBM20, RyR2, SGCD, TAZ, TCP1, TGF83, TMEM43, TMPO, TNRC1, TNNT1, TNNT2, TPM1, TTN, VCL</td>
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For this targeted gene panel testing, we extract DNA from postmortem tissues fixed in RNAlater or a blood stain card, enrich the target gene using haloplex kit and sequence the coding regions and exon/intron junction using the Illumina Miseq. We use commercial software from SOFTGenetics for sequencing data quality filtering, alignment and variant annotations, and use Sanger sequencing to test the low coverage regions and confirm reportable variants.

Variant interpretation follows American College of Medical Genetics and Genomics (ACMG) guidelines. We search the classification of a variant in clinical databases, such as ClinVar, ARVD and HGMD, to see if we agree with the variant classification submitted by others. We search pubmed for family and function studies see if there is convincing evidence of supporting the pathogenic roles of a variant. We also estimate the minor allele frequency of a variant in population databases, such as 1000 genome, ESP, and ExAc to determine if a variant is a common variant or rare. When it is unpublished, we use multiple in silico tools to predict the functional effect of variant. Finally, we incorporate the phenotype information such as ante-mortem long QT on ECG and the cardiac pathological finding of cardiomyopathy into our interpretation. Ultimately, each variant is classified as a pathogenic or likely pathogenic variant, benign or likely benign variant, or a variant of uncertain significance.

Example cases will be presented.

In conclusion, we have data to support the importance of integrating molecular testing in routine investigation of sudden unexplained death because it can explain the underlying cause of death.

As the speed of variant discovery is much faster than traditional functional studies, higher throughput functional studies are needed. In addition, family studies, such as parents-child trio studies to determine the de novo status of a variant in infants would be helpful. Finally, exome/genome based genetic study using cohort versus controls is an extremely powerful tool in identifying new disease genes.