BEYOND THE AUTOPSY: POSTMORTEM FAMILIAL VARIANT TESTING

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Postmortem genetic testing

- "molecular autopsy"
  - *Channelopathies and clotting disorders*
- More affordable, more widely available and quicker
  - *Research institutions and commercial entities*
  - *Testing results take 3-4 weeks on average*
- Identification of a pathogenic gene in individuals who die suddenly may provide a more accurate cause of death
- First degree relatives of the decedent may be at risk for having the pathogenic gene
- Testing is available, sometimes at no cost, for familial variant testing

Postmortem genetic testing

- Blood should be collected and stored in a purple EDTA tube
  - *Some labs accept blood submitted in a grey top tube; however, not all the testing can be performed on those samples*
  - *Non-frozen is preferred*
- Save 2-3 extra purple top tubes in individuals who have a sudden unexpected cardiac death, as well as those with a possible cardiomyopathy
- Bloodstain card testing can be done; however, it is not the preferred sample
- Should be submitted as soon as possible after collection

Postmortem genetic testing

- Commercial labs: full-gene-sequencing and deletion/duplication analysis using next-generation sequencing technology
  - *Pre-defined panels*
    - Cardiomyopathy, arrhythmia, skeletal muscle disease, aortopathy, connective tissue disorders, congenital heart disease, familial hypercholesterolemia and pulmonary hypertension
  - *Pathogenic or likely pathogenic variants are confirmed*
- Research institutions: Whole exome testing or targeted full-gene-sequencing may be performed
- Variants are classified according to American College of Medical Genetics (ACMG) guidelines

Variant classification

- Results compared to multiple databases and assessed with medical literature
  - *Population, disease specific and sequence databases*
- ACMG recommends a 5-tier system
  - *Pathogenic: Sequence change directly contributes to the development of disease*
  - *Likely pathogenic: Sequence change is very likely to contribute to the development of disease; however, the scientific evidence is currently insufficient to prove it conclusively*
  - *Uncertain significance: Insufficient evidence currently exists to support a more definitive classification*
  - *Likely benign: Sequence change is not expected to have a major effect on disease; however, the scientific evidence is currently insufficient to prove it conclusively*
  - *Benign: The sequence change identified does not cause disease*

Richards, et al. Standards and Guidelines for the Interpretation of Sequence Variants. Genet Med. 2015 May;17(5):405-24

Commercial cardiomyopathy panel

- Currently tests for up to 150 genes
  - *ABCC9 ACTC1 ACTN2 AGL ANK2 BAG3 CACNA1C CACNB2 CALM1 CALM2 CALM3 CASQ2 CAV3 CRYAB CSRP3 DES DMD DOLK DSC2 DSG2 DSP EMD EYA4 FHL1 FKRP FKTN FLNC GAA GLA GPD1L HCN4 JUP KCNA5 KCNE1 KCNE2 KCNH2 KCNJ2 KCNQ1 LAMP2 LMNA MYBPC3 MYH7 MYL2 MYL3 MYL4 NKX2-5 PKP2 PLN PRKAG2 RAF1 RBM20 RYR2 SCN5A SGCD SLC22A5 TAZ TCAP TGFB3 TMEM43 TNNC1 TNNI3 TNNT2 TPM1 TRDN TTN TTR VCL AKAP9 ANKRD1 CACNA2D1 CALR3 CHRM2 CTF1 CTNNA3 DTNA FHL2 GATA4 GATA6 GATAD1 GJA5 ILK JPH2 KCND3 KCNE3 KCNE5 KCNJ5 KCNJ8 KCNK3 LAMA4 LDB3 LRRC10 MED12 MYH6 MYLK2 MYOM1 MYOZ2 MYPN NEBL NEXN NPPA PDLIM3 PLEKHM2 PRDM16 RANGRF SCN10A SCN1B SCN2B SCN3B SCN4B SLMAP SNTA1 TMPO TRPM4 TXNRD2 A2ML1 BRAF CBL HRAS KRAS MAP2K1 MAP2K2 NF1 NRAS PTPN11 RASA1 RIT1 RRAS SHOC2 SOS1 SOS2 SPRED1 ACADVL ALMS1 CPT2 DNAJC19 ELAC2 MTO1 SDHA TMEM70 DEPDC5 KCNA1 KCNQ2 KCNQ3 KCNT1 PCDH19 PRRT2 SCN1A SCN8A SCN9A SLC2A1*

Commercial arrhythmia panel

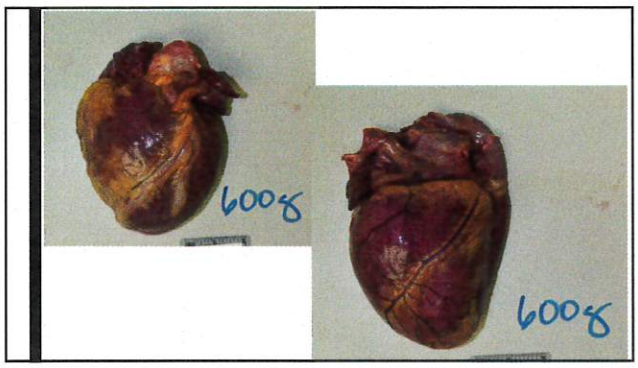
- Currently tests for up to 75 genes
  - *ABCC9 ACTN2 ANK2 CACNA1C CACNB2 CALM1 CALM2 CALM3 CASQ2 CAV3 DES DSC2 DSG2 DSP EMD FLNC GPD1L HCN4 JUP KCNA5 KCNE1 KCNE2 KCNH2 KCNJ2 KCNQ1 LMNA MYL4 NKX2-5 PKP2 PLN PRKAG2 RBM20 RYR2 SCN5A TMEM43 TNNI3 TNNT2 TRDN TTN AKAP9 ANKRD1 CACNA2D1 CTNNA3 GATA6 GJA5 KCND3 KCNE3 KCNE5 KCNJ5 KCNJ8 KCNK3 LDB3 NPPA PDLIM3 RANGRF SCN10A SCN1B SCN2B SCN3B SCN4B SLMAP SNTA1 TGFB3 TRPM4 DEPDC5 KCNA1 KCNQ2 KCNQ3 KCNT1 PCDH19 PRRT2 SCN1A SCN8A SCN9A SLC2A1*

Familial variant testing

- Can be performed on blood relatives of a decedent who was found to have a pathogenic or likely pathogenic variant on a gene or panel test
  - *Cascade screening*
  - *Available for children, parents, siblings, cousins, aunts/uncles*
  - *Looking for the abnormal pathogenic gene, regardless of the disorder it is associated with (not the whole panel initially ordered)*
  - *Family members do not need to be symptomatic*
- Saliva is the preferred sample; however, it can also be performed on blood in a purple top tube
  - *Kits can be sent directly to the individuals, so location is not a problem*
- May alleviate the need for lifelong medical tests and follow up if testing is negative

Case presentation

- 34 year old overweight black male had witnessed cardiac arrest after complaints of difficulty breathing
- Pronounced dead at the scene
- Spouse reports being diagnosed with an unspecified cardiomyopathy in another state a couple of weeks prior; prescribed a beta blocker
- Autopsy:
  - *600 gm football shaped heart with asymmetric left ventricular and septal hypertrophy with fibrosis*
  - *Myocyte disarray involving all sections*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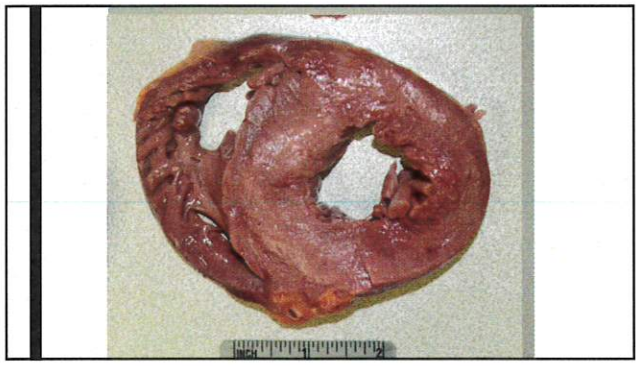
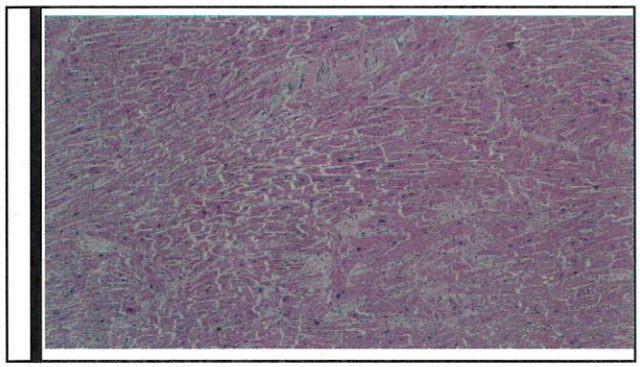
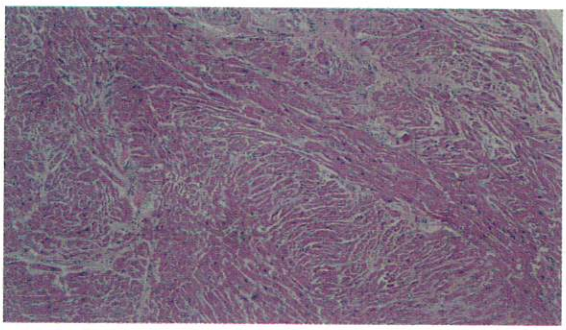
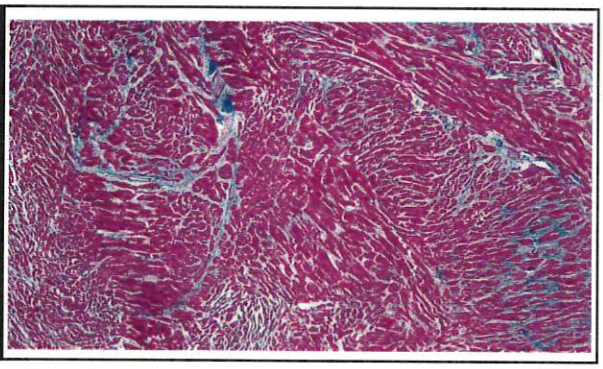








Hypertrophic cardiomyopathy

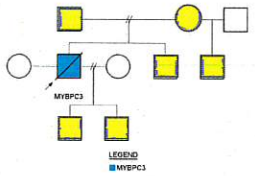
- Most common cause of sudden cardiac death in young athletes
- Variable anatomic appearance
  - *Negligible to severe myocyte hypertrophy*
  - *Minimal to excessive myocardial fibrosis*
  - *Absent to severe left ventricular outflow tract obstruction*
  - *Any of the ventricular walls may be affected*
- Variable presentation
  - *May be asymptomatic or result in sudden cardiac death at any age*
- Echocardiogram is the gold standard screening test
- No known cure; however treatments and screening tests are available
  - *Important for first degree relatives*

Hypertrophic cardiomyopathy

- First chromosomal locus identified involved MYH7 gene (encoded beta-myosin heavy chain)
- Several hundreds of mutations amongst almost 30 genes now known to exist
  - *Encode sarcomeres, calcium handling, mitochondrial proteins*
- Most common genetically transmitted form of HCM involves 9 genes encoding myofilaments critical to the cardiac sarcomere
  - *MYBPC3: One of the most common (myosin binding protein C)*
  - *MYH7 (beta-myosin heavy chain)*
  - *MYL2 (regulatory) and MYL3 (essential) myosin light chains*
  - *TNNT2 (cardiac troponin T)*
  - *TPM1 (alpha-tropomyosin)*
  - *TNNI3 (cardiac troponin I)*
  - *TNNC1 (cardiac troponin C)*
  - *ACTC (actin)*

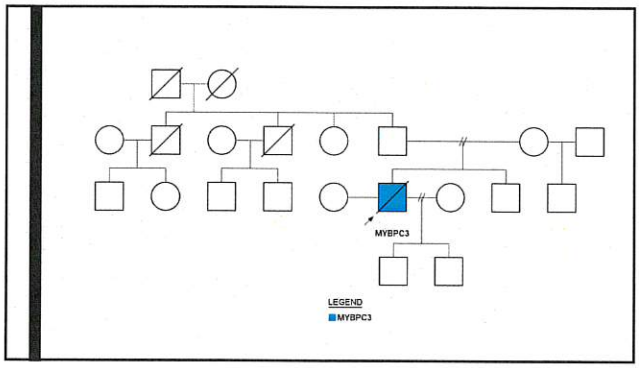
Case presentation: Genetic testing

- With consent of the family, blood sent for genetic testing for cardiomyopathy panel
  - *Pathogenic variant in MYBPC3 known to be associated with HCM identified*
- Informed the spouse of the findings and obtained more information on the family
  - *The current spouse is the second wife; no kids together*
  - *First wife has 2 kids with the decedent*
  - *Mother and father still alive; two brothers alive*
  - *Testing offered to all and all accepted*
    - Saliva is preferred sample for this testing



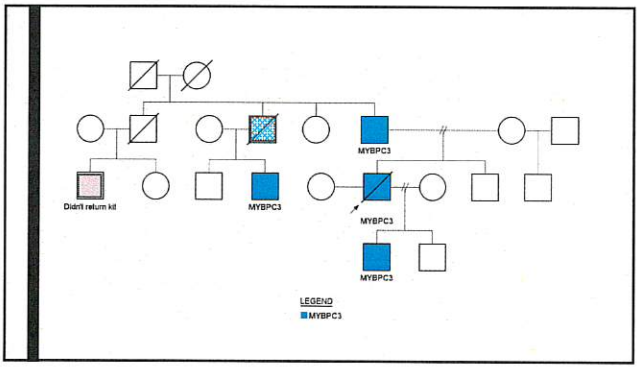
Genetic testing

- Father of decedent informed us that two of his brothers (decedent's uncles) died suddenly at young ages
  - *One had an autopsy in another state*
  - *Both brothers have living adult children*
  - *Due to the sudden cardiac death in the decedent's uncles, the decedent's cousins were located and testing was offered*
    - 11 family members total; 10 submitted samples for testing



Genetic testing results

- The decedent's father, one of his children and one cousin tested positive for the MYBPC3 mutation (sample never received for one cousin)
  - *3/10 samples positive for mutation*
- Referred for genetic counseling (offered through genetic testing lab) and referred to cardiologist with autopsy report and genetic results
  - *Father had MRI and was found to have significant left ventricular hypertrophy*
    - Internal defibrillator placed
  - *Child had full cardiac workup and has no hypertrophy at this time*
    - Will undergo cardiac testing every 2 years
  - *Cousin had echocardiogram and then referred for MRI (results not currently known)*



Postmortem genetic counseling

- Families **must** be referred for counseling services
  - *May be offered through testing lab*
  - *National Society of Genetic Counselors*
    - www.nsgc.org
    - Find a genetic counselor resource
    - Provides additional resources and information for patients and their families
- Help explain results and potential risk of transmission
- Provide emotional support
  - *Can be very difficult to receive positive results*

Postmortem genetic testing

- 14 cases submitted to date (2 year period)
  - *Gross and/or microscopic findings suggestive of cardiomyopathy, aortopathy or other sudden cardiac death*
  - *4 cases with pathogenic variants identified (28.6%)*
    - MYBPC3 (Hypertrophic cardiomyopathy)
    - TTR gene (Transthyretin amyloidosis)
    - SCN5A (arrhythmias (Brugada, long QT) and cardiomyopathy)
    - CPT2 and GAA (AR carnitine palmitoyltransferase deficiency and Pompe disease)
  - *6 cases with variant of unknown significance (VUS) identified (42.9%)*
    - 2 allowed familial variant testing
      - *Initially only 2 VUS tests offered free of charge*
      - *Contacted company and they are allowing additional tests when needed*
  - *3 cases negative (dilated cardiomyopathy (1), thoracic aortic dissection (2)) (21.4%)*
  - *1 case still pending*

Postmortem genetic testing

- Of two cases with pathogenic variants identified, 13 family members tested
  - *4 were positive for the pathogenic variant (30.7%); 1 not returned*
- Testing kits sent out for 6 other family members this month for other 2 cases
- Two cases with VUS that allowed testing
  - *3 testing kits sent out to date*
- Discussion with lab to allow testing of family members for cases with autopsy diagnosis of disease associated with the VUS mutation identified
  - *Case by case basis*

Summary

- Postmortem genetic testing may identify inheritable conditions in a decedent's family
  - *Should be standard of care for decedents identified with cardiomyopathy and/or sudden cardiac death of unknown etiology*
  - *Important not only for cause of death determination; **potential to prevent sudden cardiac death in other family members***
- Familial variant testing can be performed on first degree relatives of any age, regardless of the presence or absence of symptoms
  - *The costs and stresses of lifelong medical tests and follow up could be avoided for family members who test negative*
  - *Only tests for the pathogenic gene identified*
- Treatments and preventative measures are available for many conditions
- Family members must be referred for genetic counseling