TELLTALE HEARTS

Illustrations by Sydney Smith

Each year, tens of thousands of young people worldwide die suddenly after their hearts stop beating for no apparent reason. Genetic testing for inherited heart rhythm disorders can potentially offer grief-stricken family members an explanation for the loss of their loved ones and provide actionable diagnostic information to help them avoid the same fate. And yet, such 'molecular autopsies' are rarely performed by the forensic experts who investigate unexplained deaths. **Jeanne Erdmann** meets the medical professionals who are trying to change that.

Michelle Tipton's first hint that something was wrong came around ten o'clock in the morning, when a school administrator called to say that her son, Shannon Huber, hadn't shown up for class. Tipton had last seen Shannon the night before, when she stopped by his room to say goodnight. She left for work early the next morning without saying goodbye.

Tipton sent her father to the house to check up on Shannon, saying not to call her unless it was bad. He called and said, "It's the worst." Huber was lying in bed motionless, arms folded over his chest, his torso tipped toward the floor. The 17-year-old had died at some point during the previous night, and for a long time nobody could figure out why.

Without a clear answer, rumors took hold in the small town of Beulah, North Dakota, population 3,000, before Huber's body was even underground. This was back in March 1999, and local police were convinced that Huber had overdosed on a bad batch of drugs, perhaps crystal methamphetamine, also known as crystal meth, which was rampant in the Great Plains at the time. They treated his death as a criminal investigation. But three months later, the autopsy came back with only caffeine in Huber's system. It concluded that Huber had died of "undetermined causes." The medical examiner told Tipton and her family that they may never find answers.

Up to half of sudden so-called 'autopsy negative' deaths like Huber's leave grieving family members without a resolution—and without the knowledge that they, too, could be at risk for the same type of outcome. Unlike death from coronary artery disease, which leaves physical clues in the heart, many deaths from inherited rhythm disorders leave behind a heart that looks normal in every respect. Sometimes, the only telltale marks dwell in the genetic code.

Although medical examiners may often suspect a fatal arrhythmia, until recently, there was no way to prove it. Now, with genetic testing, heritable heart disorders can be caught after death. These 'molecular autopsies', as they're called, work backwards by looking for mutations in post-mortem tissue. In turn, those test results help guide medical treatment for surviving family members, who may unknowingly carry the same dangerous DNA variant.

But what sounds like a simple solution—genetic testing of the dead—isn't so easy. Even though molecular analyses offer a plausible cause of death for up to one-third of autopsy-negative cases in people under 40, such forensic medicine is rarely performed. There's no standardized autopsy procedure in such deaths; there's no centralized database of mutations for inherited heart problems; and there's no payer reimbursement for post-mortem genetic investigations. In many countries, health insurance coverage simply ends when the heart stops beating.

Momentum worldwide could remedy some of these issues. For example, researchers at

the Mayo Clinic in Rochester, Minnesota, are now crafting a plan that would create centers of excellence for post-mortem genetic testing where every sudden unexplained death in the young would be examined with the latest genetic technologies. And organizations such as the UK-based nonprofit Cardiac Risk in the Young (CRY) are pushing for the establishment of an official database containing all relevant mutations that could help explain why hearts sometimes suddenly stop beating.

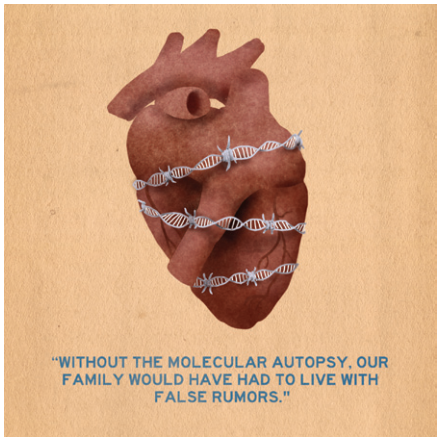
"It's critical now more than ever to share all variants found in genetic testing to help identify which variants are partly or fully responsible for sudden death," says Elijah Behr, a CRY-funded cardiologist at St. George's Hospital in London who discussed the need for a centralized database at the British Genetic Medicine Conference in Liverpool in September. "The challenge will be how to interpret all genomic data for the benefit of the patient."

Matthew Meyer, Mayo Clinic

A post-mortem proposal: Michael Ackerman wants to make molecular autopsies routine.

CSI: Minnesota

In an autopsy suite at the Mayo Clinic in July, Joseph Maleszewski reaches into a white plastic bucket and pulls out a heart taken from a young man who died suddenly of no apparent cause. The heart feels solid but not too heavy, about the weight of an apple. Over the past hour, Maleszewski, an assistant professor in the Department of Laboratory Medicine and Pathology at Mayo, has been exhibiting heart after heart in which the cause of death could be discerned by the abnormal morphology. Several of the hearts came from people who



died of diseases that weaken the heart muscle so much so that the tissue becomes stretched out. But this latest heart looks and feels normal—and that's the point. Sometimes, a death investigation needs to look beyond what's visible in a standard autopsy and down to the level of the DNA.

At Mayo, such post-mortem genetic testing has become routine for cases of sudden unexplained cardiac death. Last year, a team led by Michael Ackerman, director of the Mayo Clinic's Windland Smith Rice Sudden Death Genomics Laboratory, published a compilation of 173 molecular autopsies conducted over the course of 12 years, 45 of which yielded a presumed disease-causing mutation[1]. (This diagnostic success rate of 26% is on par with, although somewhat higher than, those observed in smaller, population-based studies from Denmark[2] and New Zealand[3].) Yet, back in 1999, when Tipton and her youngest son, Dustin Huber, traveled the 600 miles to visit the Minnesota hospital, Mayo's molecular autopsy program had barely gotten underway.

A year earlier, Ackerman and his colleagues had published a report linking a mutation in a gene called *KVLQT1* to a nonfatal incident involving a ten-year-old boy who almost drowned[4]. And in 1999, the same Mayo team described the results of a post-mortem DNA test on an apparently healthy 19-year-old woman who had died after nearly drowning[5]. She, too, carried a loss-of-function mutation in *KVLQT1*, which encodes a potassium channel protein that had previously been implicated in long QT syndrome, the most common of the inherited heart rhythm disorders, affecting 1 out of 2,500 people. The young boy's case was the first documented instance of a near-drowning serving as a catalyst to unmask the diagnosis of long QT in a large family, and the young woman's case extended the methodology into the realm of post-mortem diagnoses.

Further testing revealed that the woman's younger sister carried the same nine-base-pair deletion in *KVLQT1*, as well. As a result of the molecular autopsy and associated follow-up tests, the Mayo doctors prescribed the surviving sister a drug known as a beta blocker, which helps regulate heart rhythms and provides adequate treatment for about 80% of people with this heart condition. To Ackerman's knowledge, the sister remains alive and well today.

Ackerman used a similar approach when Tipton and Dustin arrived in the fall of 1999. They wanted the Mayo physician-scientist to tell them whether Dustin would meet the same fate as his older brother and whether there was anything they could do to prevent that from happening. This was only the third or fourth case on which Ackerman tested his theory that molecular autopsies should be performed in every incidence of sudden unexplained death among individuals aged 1 to 40, along with medical follow-ups for surviving family members.

Because Tipton's medical history included odd bouts of fainting, Ackerman again suspected that long QT syndrome might explain her son's unsolved death. Long QT can arise from mutations in a number of genes like *KVLQT1*, all of which encode essential ion channels in the heart. These mutations disrupt electrical activity, which can cause people to pass out from insufficient blood flow to their brains. Sometimes the heart corrects itself, resulting in a temporary loss of consciousness; this is not usually life threatening for individuals away from bodies of water. But other times, the ventricles beat so fast that the heart quivers and stops pumping blood altogether. Perhaps, Ackerman thought, that's what happened to Shannon Huber.

Ackerman and his colleagues put Tipton and Dustin through a series of tests designed to expose the presence of long QT syndrome—which can be difficult to diagnose because the signature prolongation of the 'QT interval', the period between the depolarization and repolarization of the left and right ventricles in the heart's electrical cycle, does not always show up on an electrocardiogram (ECG) examination. Indeed, both Tipton and Dustin had normal ECGs, even when measured over a 24-hour period. So, Ackerman's team gave them each a hormone called epinephrine, which quickens heart rates. Under this stress test, both of their hearts beat abnormally, convincing the researchers to search for a long QT mutation in the family.

They first examined Tipton's DNA. As suspected, they found a novel mutation in the *KVLQT1* gene that disrupts the function of the encoded ion channel. The Mayo group then searched Shannon's paraffin-embedded tissue and confirmed he carried the same mutation. Dustin, the younger brother, had it as well. Ackerman and his colleagues published the findings in 2001 in *The American Journal of Forensic Medicine and Pathology*[6].

"Without the molecular autopsy, our family would have had to live in this community with false rumors that people believed," Tipton says. Soon after the diagnosis, she and Dustin started taking beta blockers. Both also opted for implantable cardioverter-defibrillators, or ICDs, devices that continually monitor heart rhythms and deliver electrical pulses when needed to correct dangerous arrhythmias. Dustin's ICD shocked his heart two weeks later. "That's how close it was," Tipton says.

Heartbreak averted

Shannon Huber's story exemplifies the need for molecular autopsies. Here was a tragic death that, thanks to post-mortem DNA testing, yielded a diagnosis that prompted preemptive strategies, ultimately preventing further tragedy from striking Tipton and her family.

Ackerman can rattle off many more successes like this. In 2002, for instance, his team published an effort in which they used genetic analyses, including a molecular autopsy, to identify a mutation in *TPM1* in a family affected by hypertrophic cardiomyopathy (HCM), a disease of the heart muscle tissue[7]. The family had already lost a six-year-old son to the disease and had sought out Ackerman's help after the sudden death of their eight-year-old daughter. By pinpointing the causative mutation, the Mayo doctors could reasonably guide treatment decisions for the two surviving children in the family. The older son carried the HCM-causing mutation and received an ICD for disease prevention. The younger daughter had a normal *TPM1* variant and did not need further intervention.

Of course, not all molecular autopsies come back with such actionable results. And even when they do, not all experts agree over how to proceed with that information. ICDs, for example, can introduce serious complications, such as inappropriate shocks or device-related infections. So, opinions vary on whether all people at risk of long QT syndrome should

Only in New York: Mysterious deaths routinely solved with DNA

NEW YORK — In the Kips Bay neighborhood of Manhattan, near the East River and an easy walk from the UN headquarters, sits the Office of the Chief Medical Examiner (OCME) medical genetics laboratory, the only one of its kind in the US. The laboratory opened for business in 2005 and is devoted to identifying unexplained diseases in the dead. It's separate from the crime lab that provides DNA testing for law enforcement cases involving, for example, rape, assault and murder—although sometimes the two labs have to work together. According to Jennifer Hammers, a forensic pathology specialist at the OCME, the office once used post-mortem DNA testing to clear a husband of wrongdoing after his wife, who was found later to harbor a genetic mutation that causes long QT syndrome, died after falling and hitting her head, presumably as a consequence of a disease-related fainting episode.

Jeanne Ekstrom

Special victims unit: Yingying Tang presides over New York's medical genetics lab.

Benches and equipment in this facility are so free of clutter that it's difficult to believe that lab director Yingying Tang and her crew of six process nearly 500 DNA samples each year. Before entering the lab, every visitor dons a lab coat and gloves and takes one additional step. All non-employees swab the insides of their cheeks with two cotton buds. These go into a three-by-five-inch manila envelope labeled with name, title and the word 'visitor'. DNA from those samples is then sequenced and stored for one year, in the event that anyone unwittingly sheds genetic material during the tour.

The idea for a medical genetics laboratory came about thanks to Charles Hirsch, often called the 'father of forensic pathology', who served as chief medical examiner for New York from 1989 until earlier this year. "We are so extraordinarily fortunate," says Barbara Sampson, who became acting chief medical examiner when Hirsch retired in February. "Genetic tests are prohibitively expensive, and most medical examiners don't have that luxury." Funding for the medical genetics facility comes from City Hall, with additional support provided by the National Institute of Justice, the research arm of the US Department of Justice.

Tang ensured that the laboratory received and maintains accreditation from the College of American Pathologists, which puts the quality of molecular autopsies done here on par with clinical diagnostic laboratories in hospitals. She also develops every post-mortem genetic panel, spanning a variety of conditions, including heart rhythm disorders, deep vein thrombosis, sickle cell disease and blood clotting disorders.

Once Tang's crew finds a dangerous mutation for any genetic disease, the medical examiner's office notifies the family or their physician. According to Sampson, cause of death is determined in about 10–15% of cases, which isn't bad, she says, when you consider that "we would have solved zero cases without this technology." —*JE*

have ICDs implanted, especially given that only around 10% will have sudden death as their first symptom, whereas an estimated 30% will live out their lives and never experience any problems.

For other heart rhythm disorders, such as Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia, which together account for around a quarter of all sudden cardiac fatalities, death is more often the first and only sign. Yet, even for these diseases prophylactic ICD therapy remains controversial. "This not an easy business, I can tell you," says Mitchell Faddis, head of cardiac electrophysiology at the Washington University School of Medicine in St. Louis, Missouri.

Making matters worse, genetic tests often come back with variants of uncertain significance—"genetic purgatory," as Ackerman puts it. Deciding whether such mutations are harmless or harmful is sometimes impossible, which raises anxiety levels in surviving family members. Doctors don't want to run ahead of scientific knowledge by providing medical treatment to families on the assumption of ambiguous test results or by telling the family they have these diseases when they don't, mistakenly making them think they're living with a ticking time bomb.

"We may save lives with this technology, but we may also harm people with this technology," says Phillip Cuculich, who helped launched a new Cardiovascular Genetics Clinic at Washington University School of Medicine in July of this year. "In our business we can't be wrong, and that's the hard part about this."

Molecular autopsies can also bring a host of legal and ethical complications. For example, unlike in a clinical context, for post-mortem studies most countries do not require forensic pathologists or medical examiners to seek permission from relatives of the deceased to conduct DNA analyses, nor do they have to notify family members of the results of any tests undertaken. That needs to change, says Bernice Elger, head of the Institute for Biomedical Ethics at the University of Basel in Switzerland. She argues that families should at least be informed that treatable genetic disorders can be identified; they can then decide for themselves whether to learn specific test results or to undergo follow-up testing.

To help address some of these issues, the Mayo Clinic team is proposing a standard set of protocols to enable what Ackerman titles the 'comprehensive cardiac autopsy'. This would marry all available genetic resources—including whole-exome sequencing, which decodes the entire protein-coding portion of the genome—and could be done on frozen tissue or blood samples from all young sudden death victims.

In work presented at last year's American Society of Human Genetics meeting in San Francisco, Ackerman, Maleszewski and their colleagues presented a proof-of-principle case report involving a 16-year-old who had died in her sleep. The victim's exome revealed a previously undocumented mutation in the *MYH7* gene, which encodes a subunit of myosin and is involved in cardiac muscle contraction. Mutations in *MYH7* account for around 40% of cases of HCM. Based on the analysis and further evaluation of the victim's heart tissue, the Mayo researchers concluded that this disease killed the otherwise healthy adolescent.

Ackerman wants to begin his comprehensive cardiac autopsy effort as a US-wide initiative but hopes it eventually takes off worldwide. To help endow the proposal, Ackerman suggests a combined funding strategy involving multiple sponsors, including sudden death advocacy organizations, private philanthropists and the US Department of Health and Human Services (with possible contributions from the Centers for Disease Control and Prevention and the National Institutes of Health). His ideas are starting to gain support. In June, for instance, the US National Association of Medical Examiners published a white paper outlining guidelines for how medical examiners should collect and store DNA-friendly tissue samples in autopsy-negative deaths involving young people[8].

Payable on death

While the idea of molecular autopsies has gained traction, the question of who will pay for them remains a key roadblock. In the absence of a national program to cover the cost of molecular autopsies, bereaved families are often left to pay for these tests out of pocket. "It's a very difficult situation when the person who needs genetic testing is deceased because then they are not insured," says Sumeet Chugh, director of clinical electrophysiology at the Cedars-Sinai Medical Center in Los Angeles—"which is ironic," he adds, "because there are a lot of people in a given family that could be depending on that information." (New York City is one exception, where the Office of the Chief Medical Examiner routinely conducts molecular autopsies on sudden unexplained deaths; see 'Only in New York'.)

Clinicians elsewhere have succeeded in getting governments to pay for post-mortem genetic testing—usually through divisions of justice, although sometimes with support from ministries that cover healthcare. After the unexplained death in 2001 of a 12-year-old boy just five minutes into a field hockey game, Jon Skinner and his colleagues convinced the New Zealand Ministry of Justice to pay for DNA testing of every unexplained case of sudden death under age 40. "To me, this is a no-brainer," says Skinner, a pediatric cardiologist at Starship Children's Hospital in Auckland. In 2008, the Ontario Forensic Pathology Service in Toronto likewise opened a molecular autopsy laboratory in which several government ministries now pay for post-mortem genetic testing.

Better therapeutic strategies could come from the large sample sizes provided by such routine molecular autopsies. For example, such research may help explain why some mutations are ultimately fatal while others can be safely ignored. But most importantly, says Skinner, genetic testing of the dead can provide families with closure for a past tragic event and a sense of control going forward. "The valuable lesson here is that genetic testing can give a diagnosis when one was not available, and one that helps us focus on looking for a single condition in surviving relatives to protect them," says Skinner. "Families are so grateful when you can give them an answer."

Jeanne Erdmann is a science writer based in Wentzville, Missouri. ***Reporting for this story was supported by a fellowship from the Association of Health Care Journalists, with assistance from the Commonwealth Fund.***

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