Tracking Down Mutations That Can Stop the Heart

SPECIAL FOCUS: CARDIOVASCULAR DISEASE

With heart disease the leading cause of death in developed countries, *Science* is taking a special look at two factors that have received little publicity. One story deals with mutant genes that cause frequently fatal heart defects, while the other discusses recent results linking infections to artery-clogging plaques

People were shocked when Boston Celtics star Reggie Lewis collapsed on the basketball court in the summer of 1993, but the death also had a familiar ring. Every few years a well-known young athlete drops dead without warning during a sporting event, victim of an undetected genetic heart condition. These cases provide an all-too-graphic demonstration that heart disease doesn't have to begin in midlife with the development of clogged arteries but can arise from stealthy inherited flaws in the heart itself. "People have known for quite a while by looking at families with heart disease that genetics plays a role" in conditions ranging from congenital heart malformations to fatal disturbances in heart rhythms, says geneticist Mark Keating of the University of Utah Health Sciences Center in Salt Lake City.

But until 8 years ago, none of the genes at fault had been identified. Then, Keating says, came "an explosion of information" about mutations that directly impair the heart—an explosion that still hasn't let up. In 1990, Christine and Jon Seidman at Harvard Medical School in Boston and their colleagues found the first mutation, in myosin, a key protein in heart muscle. Since then, discoveries of heart-handicapping mutations have been pouring out of numerous labs at an ever-increasing rate, yielding more than 100 mutations in more than a dozen genes.

Some, like a new gene reported on page 108 by the Seidman team, affect the formation of the heart structure itself. Mutations in these genes cause defects such as an abnormal hole in the wall, or septum, that divides the upper two chambers of the heart. Other mutations disrupt ion channels, the protein pores that control the electrical conductance of heart muscle, altering the heart's normal rhythms. And mutations in myosin and other proteins involved in muscle contraction are at the root of most of the hereditary cardiomyopathies, conditions that cause the heart's walls either to thicken abnormally or to stretch out.

Cardiologists are already putting this new information to work to design genetic tests that will identify those at risk of dying from the heart defects, as well as treatments that will safeguard their lives, such as drugs tailored specifically to reverse a patient's genetic flaw. And the benefits may not be limited to the hereditary defects.

Although these conditions are relatively rare—the most common strikes only 0.2% of the population—many are similar to much more common heart ailments that develop later in life. For example, disturbances in heart rhythms, which can kill very quickly, often occur in people who have suffered heart attacks or who take certain medications. "What we have learned [about the genetic conditions] may be able to translate to the much bigger problem of sudden cardiac death," says Michael Ackerman, a cardiology fellow at the Mayo Clinic in Rochester, Minnesota.

SOME OF THE GENES THAT CAUSE HEART DEFECTS

Category	Condition	Gene	Function
Developmental disorders	Holt-Oram syndrome	*TBX5*	Transcription factor
	Atrial-septal defect	*NKX2-5*	Transcription factor
Cardiomyopathy	Hypertrophic cardiomyopathy	β myosin heavy chain	Muscle contraction (force generation)
	"	myosin essential light chain	"
	"	myosin regulatory light chain	"
	"	troponin T	"
	"	troponin I	"
	"	cardiac myosin-binding protein C	"
	"	α tropomyosin	"
	Dilated cardiomyopathy	dystrophin	Muscle contraction (force transduction)
	"	actin	"
Arrhythmias	Long QT syndrome	*KVLQT1*	Potassium channel
	"	*HERG*	"
	"	*minK*	"
	"	*SCN5A*	Sodium channel
	Brugada syndrome	*SCN5A*	Sodium channel

Holes in the heart

Few heart flaws are more obvious than a hole, or a set of holes, between the two upper chambers known as atria. Gene flaws aren't always to blame; some babies are born with a small defect because a hole that is normally present in the fetal heart didn't close up in time. Such defects often disappear on their own within weeks or months.

But some babies, perhaps as many as 1 in 1500, are born with a hole that is larger than it should ever have been during fetal life, or with multiple holes that make the septum look like Swiss cheese. These atrial-septal defects (ASDs), which usually have to be repaired surgically, don't just result from a timing problem, but are "truly malformations" caused by errors in early development, says Christine Seidman.

Some of these developmental errors run in families, and in two cases researchers have discovered the genetic cause. Last year, the Seidman team and a group led by David Brook of the University of Nottingham in the United Kingdom reported that they had found the mutant gene responsible for Holt-Oram syndrome, a rare hereditary condition that causes holes between the atria and sometimes the lower chambers of the heart, known as ventricles, as well as arm and hand defects. The culprit gene, *TBX5*, encodes a transcription factor, a protein that regulates other genes, including some of those needed to build a normal heart.

In the work reported in this issue, the Seidmans describe a similar gene that, when mutated, causes another form of ASD, one not accompanied by the arm and hand problems characteristic of Holt-Oram. This gene, too, encodes a transcription factor; it is the human counterpart of a fruit fly gene called *tinman* because, like the Tin Man in *The Wizard of Oz*, fruit fly embryos that lack both copies of the gene have no hearts at all. As with *TBX5*,

humans missing only one copy of *tinman* have heart defects that include ASDs.

Identification of these genes may help solve a critical puzzle, says Seidman: "Adults with ASD appear to die at an earlier age than do normals," even when the hole is successfully repaired. They apparently have a higher risk for sudden death from heart block, a blockage of the electrical signal that travels through the heart muscle and controls the heartbeat.

For years physicians assumed that the conduction problem at the root of the heart block results from the repair surgery, but Seidman notes that surgery to repair the far more common, nonhereditary holes does not increase the risk of heart block. "It clearly is a component of the genetic defect," she says, adding that a better understanding of the transcription factors and the genes they regulate may reveal how the mutations lead to heart block. And that in turn may suggest less invasive ways to prevent it.

Currently, heart block is best prevented with an implantable pacemaker, provided it's detected in time. "We need to be more careful in monitoring patients [with mutations] for the development of heart blocks," Seidman says, so that they can receive pacemakers before a heart block kills them. Now that the affected gene is known, it may become possible to screen infants in families with the mutation to ensure that no cases are missed.

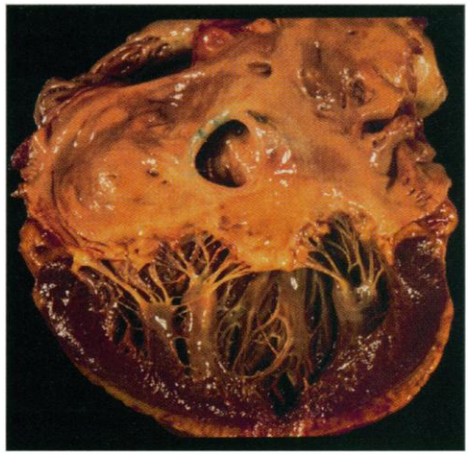
The thick and the thin

Not all genetic heart conditions are evident at birth, as the ASDs are. Roughly 1 in 500 people harbor subtle genetic errors that cause cardiomyopathies, afflictions of the heart muscle that don't become apparent until adolescence or even adulthood. Hypertrophic cardiomyopathy (HCM), the leading cause of sudden death in young athletes, was blamed for the deaths of basketball stars Hank Gathers of Loyola Marymount College in 1990 and the Celtics' Lewis. The condition causes cardiac muscle cells to grow larger, thickening the heart's muscular wall. The affected heart pumps strongly but doesn't relax well for the filling phase of the heartbeat. In contrast, dilated cardiomyopathy (DCM) produces a stretched-out, thin-walled heart that fills with excess blood but can't efficiently pump it out.

Both conditions can lead to the uncoordinated fluttering of the heart called fibrillation, which pumps no blood and is fatal if not halted quickly. And it turns out that both can be caused by mutations in genes encoding proteins, such as myosin, that play a role in heart-muscle contraction.

The mutant myosin gene the Seidman team identified in 1990 causes one form of HCM. Before that work, "it was always thought that the hypertrophy was the primary defect, that there was something wrong with the structure of the heart," says researcher Ketty Schwartz of the French biomedical research agency INSERM in Paris, whose lab also studies myopathy genes. But the Seidmans found that the mutation actually changes the gene encoding the heavier of the two protein chains that make up the myosin molecule. The discovery that a mutant contractile protein was at fault "was a big surprise," says Schwartz. "No one thought that the contractile proteins were altered."

The finding was no fluke, though. It was soon followed by a flurry of confirmations, in which other teams found mutations in the myosin heavy chain in families with HCM. Subsequently, mutations affecting six other proteins that work together to make the muscle contract—the two myosin light chains, tropomyosin, troponin T, myosin-binding protein C, and troponin I—turned up in HCM patients. At last count, 109 mutations in these seven genes had been shown to cause HCMs, says cardiologist Robert Roberts of Baylor College of Medicine in Houston.



FREDERICK J. SCHOEN/BRIGHAM AND WOMEN'S HOSPITAL AND HARVARD MEDICAL SCHOOL

Holey heart. Mutations in heart-specific transcription factors can cause defects such as this hole in the wall between the heart's atria, or upper chambers.

The discovery of these mutations and the characterization of the diseases they cause, which is going on in several laboratories, including Schwartz's and the Seidmans', could be a big help in diagnosis. For example, troponin T mutations cause a particularly virulent form of HCM, with high rates of sudden death, but little hypertrophy. That means cases may be missed until fibrillation strikes. "We have these horrific stories where kids who are unrecognized to be affected are dying," says Christine Seidman. She thinks it may be worthwhile to screen children in afflicted families, and implant defibrillators in those with the mutant gene.

The mutations in myosin-binding protein C could also aid in diagnosis. These turn out to cause a much milder form of HCM that doesn't strike until age 50 or so and previously wasn't considered to be genetic. "We frequently see [older] people with hypertrophy," says Roberts, and some of them don't have high blood pressure or any other risk factors for the condition. "We now suspect that probably that is a familial form." Indeed, Christine Seidman's group has already found myosin-binding protein C mutations in such cases. The discovery should enable affected families to take measures to reduce their risk of sudden death.

Researchers have also made progress in understanding how mutations in at least two of the contractile proteins might cause the heart to hypertrophy. Muscles contract when filaments of myosin slide over filaments of actin, causing the muscle cells to shorten. In test tube studies performed in 1992, physiologist H. Lee Sweeney and his co-workers at the University of Pennsylvania, Philadelphia, found that the mutant form of myosin interferes with this sliding. "The [mutant] myosin put a drag on the normal myosin and slowed everything down," Sweeney says. "We predicted it would greatly drop the power output of any muscle that incorporated it."

Those predictions were confirmed when he teamed up with Neil Epstein and Lameh Fananapazir of the National Heart, Lung, and Blood Institute to look at samples of muscle with the myosin mutation. "The muscle shortened very slowly," Sweeney says, "the power output was diminished greatly, and the force was down somewhat as well." Mutations that cause the most severe power loss also caused the most severe disease. That suggests, he says, that the cardiac muscle cells enlarge to compensate for this reduced power.

Some of the disease-causing mutations in troponin T have just the opposite effect, according to Sweeney's team and that of Larry Tobacman, a biophysicist at the University of Iowa College of Medicine in Iowa City: They make the actin slide past myosin faster than usual. Cells with the mutation "generate reasonable power output," says Sweeney, and that may explain why the patients' hearts show little hypertrophy. But, he says, "the fact that [the contractile machinery] is cycling so fast is a big problem, because now you are using a lot more energy." This, he suggests, could cause a local energy shortage when the heart is under stress and might trigger arrhythmias.

As for DCM, the other major form of cardiomyopathy, its most common cause is poor blood supply to the heart muscle, which kills off many of the heart's muscle cells, causing it to stretch and weaken. But 25% of the cases may be genetic. Some of these occur in people with muscular dystrophy, in which the heart, along with other muscles in the body, lacks dystrophin, a protein that transmits the force of the contraction to the

protein scaffolding outside the cell.

Two DCM mutations found earlier this year by Utah's Keating seem to have a similar effect. Both strike the gene for actin, which connects myosin to dystrophin and other proteins at the cell membrane. Unlike the mutations that cause HCM, says Keating, "these mutations don't cause a problem with force generation; they cause a problem with force transmission."

That inadequacy of force transmission may put deadly stress on cardiac muscle cells in times when the heart is working hard, says Keating, and over time that could lead to the death of muscle cells seen in DCM. Other mutations that affect energy metabolism also cause DCM, and defective energy metabolism also can cause cell death. "It may be that what happens in dilated cardiomyopathy is that there is just a lot of cell death," says Jon Seidman. That may explain why its genetic causes are more diverse than the causes of the other syndromes, he adds, because "lots of different things can kill a cell."

Unhealthy rhythms

Whether the primary problem is a hole in the heart or a mutation in myosin, what ultimately kills people with these conditions is an arrhythmia, caused by electrical conduction glitches in the heart muscle. A third class of genetic conditions causes these conduction problems directly. The best studied of these syndromes is long QT syndrome (LQTS), which is named after the characteristic change it produces in a patient's electrocardiogram: a lengthening of the QT interval, the time in the heartbeat when the heart muscle is recovering from one contraction, before it can be triggered to contract again.

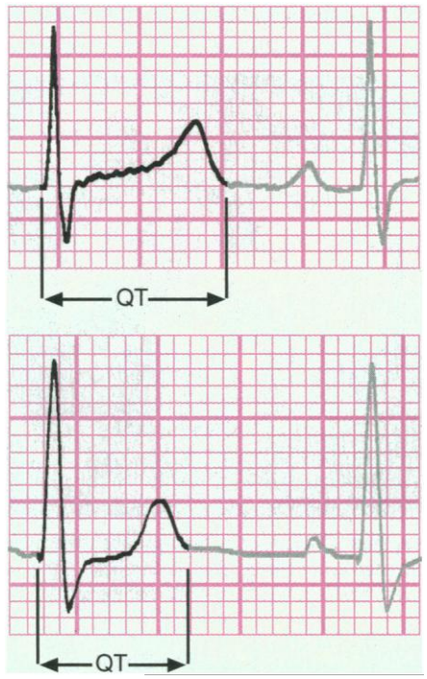
The defect is dangerous because it not only lengthens the recovery time but also makes it more variable from cell to cell. Normally, the electrical impulse that causes the heart to contract sweeps in a regular fashion through the heart muscle from top to bottom, but in LQTS it may cycle backward, forming little eddies of current that interrupt the heart's rhythm and can send the heart into fibrillation and sudden death.

LQTS can be triggered by a variety of nongenetic causes, ranging from alcoholism to some prescription drugs. Hereditary long QT only affects 1 in 10,000 people, but for those people, the risk of death can be 50% over 10 years. And because young, healthy people don't routinely get electrocardiograms, LQTS often goes undiagnosed. In one-third of the people who die of LQTS, "their death is their first and last symptom," says Ackerman, of the Mayo Clinic.

In the mid-1990s, Keating's team identified four mutant genes that cause LQTS. All four encode ion channels, the protein gateways that allow charged ions to flow through a cell's membrane. The finding makes biological sense, because the ion flows controlled by these channels produce the action potential—the wave of electrical activity that triggers muscle contraction.

The action potential begins when sodium channels open, allowing positively charged sodium ions to rush into the cell. This reverses the charge across the membrane, making its inner surface momentarily more positive than the outside. That condition, known as depolarization, shuts the sodium channels and after a delay opens potassium channels, which allow positively charged potassium ions to surge out of the cell, returning the membrane to its resting state so that the muscle can contract again.

Potassium channels are "the key off switches for the heart" that end the action potential, says Keating. And three of the four mutations his team identified, underlying at least 80% of the inherited cases of LQTS, are in potassium channel genes. These mutations reduce the number of working channels in the patients' heart cells, thereby delaying the end of the action potential and lengthening the QT interval.



Dangerous delay. As these electrocardiograms show, the heart of a patient with long QT syndrome *(top)* takes 0.08 millisecond longer to recover from an action potential than a normal heart *(bottom)*.

Sodium channel mutations can also cause LQTS. Some mutations result in channels that stay open even after the cell membrane is depolarized, allowing sodium to go on leaking into the cell, where it prolongs the action potential. Another mutation has the opposite effect, decreasing the flow of sodium ions into heart cells and shortening the action potential. That condition, known as Brugada syndrome, also leaves the heart more susceptible to the current eddies that cause fibrillation. It is a major cause of death of young men in Asia—in some countries second only to auto accidents.

The revelation that mutant ion channel proteins underlie hereditary arrhythmias has already changed how physicians treat these conditions. "Understanding precisely the mechanism allows us to target the therapy very specifically," says Johns Hopkins University cardiologist Gordon Tomaselli. For example, in LQTS patients with the sodium channel mutation, some physicians are using a drug called mexiletine, which blocks the leaky channels. "Lo and behold," says molecular cardiologist Jeffrey Towbin of Baylor, "if you look at electrocardiogram before and after, the before shows severe long QT syndrome and the after shows near-normalcy." Likewise, cardiologists have been prescribing potassium supplements for people who have potassium channel mutations to get more ions through the existing channels, although Tomaselli notes that researchers have yet to show that this improves the prognosis.

Some commonly prescribed antibiotics, antihistamines, and antifungal agents increase the chance of heart arrhythmias, and discovery of the channel defects has made it clear as to why. Most of these drugs block HERG, a potassium channel that is mutated in about 30% of LQTS cases. Researchers studying HERG have learned, says Ackerman, that this channel normally acts as a fail-safe: It quickly opens to offset inappropriate electrical signals that could be the trigger for fibrillation. The fact that the drugs as well as mutations inhibit HERG "puts the inherited and acquired forms [of LQTS] ... in a common pathway," says Ackerman.

Heart researchers hope their understanding of all these mutations will reveal more of the common physiological pathways that link genetic causes of cardiac death to their nongenetic counterparts. Then they will be in a much better position to keep hearts—whether flawed by genes or damaged in later life—from suddenly stopping. **–MARCIA BARINAGA**

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