# A CANCER LEGACY

## Once viewed as tragic anomalies, many childhood cancers may have their roots in inherited mutations

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Two cancers in a young family: It was either horribly bad luck or the tip-off to a deeper connection. The story unfolded when Michael Walsh, a young pediatric oncologist, was caring for a boy undergoing a bone marrow transplant at St. Jude Children's Research Hospital in Memphis, Tennessee. The child wasn't responding as expected to standard therapies, suggesting something curious about the rare form of leukemia from which he suffered. Suspicious, Walsh inquired about the family's health history—and was startled to learn that the boy's father had died of brain cancer a couple years earlier, at age 31.

Walsh took a skin biopsy from the child and reached out to MD Anderson Cancer Center in Houston, Texas, where the father had been hospitalized. There he tracked down a stored sample of noncancerous tissue from the man's brain. Sequencing DNA in father and son's normal cells revealed a defective gene, *P53*, passed from one to the other. Crucial for DNA repair, *P53* is a familiar villain in cancer. Mutations in it cause Li-Fraumeni syndrome,

After 8-year-old Claudia was diagnosed with a soft tissue cancer, her mother Amanda Seymour learned that the disease and a family history of thyroid problems were tied to a gene mutation.

which predisposes people to many pediatric and adult cancers. The boy's form of leukemia, however, was not on that list. "Was this driving the cancer the boy had?" Walsh, now at Memorial Sloan Kettering Cancer Center in New York City, remembers wondering. "It certainly smelled like it … [but] we didn't really know what to think."

Hospitals increasingly experiment with broad DNA sequencing of tumor cells, a fishing expedition of sorts to try to pinpoint a cancer's genetic flaws and guide treatment. Until recently, however, they rarely did the same for DNA in other tissues, such as the boy's skin cells, which might reveal cancer-causing mutations that the patient was born with. There were a few reasons: Those mutations are exceedingly rare, the thinking went, and it would be easy to identify patients whom sequencing might help based on their form of cancer.

Those suppositions are slowly being called into question—at least when it comes to children. In the last several years more than 2000 youngsters with cancer, from infants to young adults, have had the DNA in their noncancerous cells run through sequencing machines. The readouts are casting tantalizing clues across the pediatric cancer world, and changing how doctors think of the disease.

Unlike an adult, who may have spent decades accumulating genetic abnormalities and suffering the effects of poor diet, smoking, and other environmental factors, a 3-year-old with cancer presents a conundrum. "Up until 5 or 6 years ago, many, many people thought [such cancers] were just mistakes of nature," says John Maris, a pediatric oncologist at The Children's Hospital of Philadelphia (CHOP) in Pennsylvania. Yet others, including Maris, had long suspected that inherited mutations might play a considerable role. Changes in a handful of genes, such as *P53* and *RB*, which is linked to the eye cancer retinoblastoma, have long been considered culprits in rare childhood cancers. But for most affected kids, "we just didn't know" which genes might be at work, says Will Parsons, who treats brain tumors at Texas Children's Hospital in Houston.

A deeper dive into the DNA of young cancer patients is now turning up evidence that a sizable subset of childhood cancers might be rooted in inherited genes, or mutations so soon after conception that they pervade every cell—rather than in freak genetic events that accumulate after birth and turn cells malignant. And the suspect genes are not necessarily the expected ones. A gene mutation that predisposes men to prostate cancer has shown up in a child with a kidney tumor; defective genes previously associated with breast cancer are appearing in children with the nerve tissue cancer neuroblastoma. These cases and many others suggest that the silos into which cancer genes have been neatly organized may not be so clear-cut.

"We've been taught as pediatric oncologists never to test these genes in children because they only predispose" to adult cancers, something that's potentially wrong, says Kim Nichols, a pediatric oncologist who was recruited from CHOP to St. Jude a year ago to lead the hospital's new cancer predisposition clinic. The findings engender hope that some cancers, either in children who survive their first bout with the disease or in their siblings and parents, could be prevented or caught early. But they also raise complicated questions: about what to communicate to families in the midst of a health crisis, whether to screen other family members for certain mutations, and what to do if those relatives share them.

**ADVANCES IN PEDIATRIC SURVIVAL** are justly hailed as a shining success in the wider "War on Cancer," but most of the gains involve the commonest form of childhood leukemia and solid tumors that haven't spread. Across many pediatric cancers, survival rates have plateaued. "We really need to use new technology and new biological insight to improve the cure rate," says Rajen Mody, a pediatric oncologist at the University of Michigan (UM) and C.S. Mott Children's Hospital in Ann Arbor, who often works with families whose children are out of options. Like many shifts in medicine, Mody says, the desire to sequence normal tissue was born "out of frustration, or necessity."

There was reason to hope this DNA might hold some answers. Anecdotally, doctors often see cancer's tentacles stretching through families with affected children. "You recognize it," says David Malkin of The Hospital for Sick Children in Toronto, Canada. Malkin guesses that about 40% of children in the hospital's cancer clinic have a family history suggesting a faulty, inherited gene. A study done in the cancer survivor clinic at Cincinnati Children's Hospital Medical Center in Ohio supports that estimate. Detailed family histories of 370 youngsters suggested that 29% of them might have cancers with an inherited genetic component. "And I think that's a gross underestimate," says pediatric oncologist Joshua Schiffman, who himself survived Hodgkin's lymphoma as a teenager and now works at the University of Utah in


Recuperating at home after cancer treatment, Claudia works on some homework while surrounded by craft supplies. She will turn 9 next month, and her younger brothers are being tested for the gene mutation she shares with her mother.

Salt Lake City. Many children who inherited the most aggressive gene mutations, he says, likely died and went uncounted in this sample of survivors.

Mody was among the first to embark on a serious hunt for inborn mutations. In 2011 he met Arul Chinnaiyan, a UM pathologist who was exploring a new approach to sequencing in adult cancer patients. Sequencing the tumor—itself a relatively new strategy, though an increasingly popular one—can't always unveil which mutations the patient was born with, because cancer transforms a genome into something unrecognizable, overflowing with abnormal DNA. Instead, Chinnaiyan combined sequencing tumor DNA with reading the DNA of normal cells in men with prostate cancer, to see whether he could learn more about their disease. Mody wanted to do the same for his young patients. "This would be the time," he told Chinnaiyan. "Let's try this."

They recruited 102 youngsters, most of them fitting the profile Mody typically sees: children and teenagers whose cancer has spread and whose disease isn't responding to standard therapy. On average they were ten-and-a-half years old. At a cost of about $5000 per patient, the team sequenced and analyzed the "exome," the DNA that produces proteins, in both tumors and healthy cells.

Ten percent of the young participants had been born with a mutation in a cancer gene—but sometimes one linked to cancers very different from the child's disease, Mody, Chinnaiyan, and their colleagues reported in the *Journal of the American Medical Association* (*JAMA*) in September 2015. A 4-year-old with neuroblastoma had healthy cells harboring a mutated version of *BARD1*, a gene associated with breast cancer; a child with an abdominal mass had an abnormal version of *MITF*, which predisposes to melanoma and renal cancer in adults. Despite significant uncertainty about whether these faulty genes were driving the children's cancers, families were referred for genetic counseling and additional testing, if they chose.

A similar crisscrossing of cancer genes is showing up in older patients. In November 2015, a group from Sloan Kettering reported in *JAMA Oncology* that 12% to 15% of 1566 adult patients with advanced cancer had cancer gene mutations in healthy cells. Patients with stomach cancer, a neuroendocrine tumor, and a sarcoma had mutated versions of the breast cancer genes *BRCA1* or *BRCA2*. An individual with colon cancer had a pervasive *RET* mutation, which is normally found in those predisposed to thyroid tumors. "There were adults with cancer with mutations in genes that would never have been reported and tested" under normal circumstances, Nichols says.

What these unexpected mutations actually mean can be unclear. They could be flukes, entirely unrelated to the cancer at hand. And then there are the children whose normal cells have variants of unknown significance in known cancer genes, which may or may not have anything to do with their disease. "What are we going to say to patients about this?" says Stephen Chanock, a pediatric oncologist at the National Cancer Institute (NCI) in Bethesda, Maryland, who is sequencing 6000 exomes from childhood cancer survivors. One way to learn more is by heading back to the lab and testing, in a petri dish or a mouse, whether a particular mutation makes cells more vulnerable to the patient's particular cancer.

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> **Kim Nichols**, St. Jude Children's Research Hospital

Another is to accumulate more examples in patients. Because pediatric cancer is relatively rare, that can be difficult—but it also means that just a few cases can signal a link. At CHOP, Maris has found mutated versions of *BARD1* in several children with neuroblastoma, two of whom he reported in a recent paper. Neuroblastoma is diagnosed in only about 700 children in the United States each year. "You see this [mutation] in four, five, six neuroblastoma patients," Maris says. Especially when it doesn't turn up in children without cancer, it's hard to deny a connection. "Yeah, I'm pretty convinced."

**FOR FAMILIES**, the knowledge that an inherited mutation could have contributed to a child's cancer can be unnerving. "Initially I was like, this is stress I don't need, there's a feeling of guilt, you start thinking 'I gave this to my child,'" says Amanda Seymour, an attorney in Biloxi, Mississippi. Last spring, while she was trying to wrap her mind around her 8-year-old daughter Claudia's diagnosis of rhabdomyosarcoma, a soft tissue cancer, Seymour found herself sitting opposite a genetic counselor at St. Jude. Her own family had a history of thyroid problems stretching back generations—a pattern so striking that they were the subject of a scientific paper in the 1980s. Seymour herself had had benign thyroid growths removed, as had two other siblings, and her older sister, now 44, was diagnosed with thyroid cancer at age 3. When combined with a cousin's rare kidney disorder in early childhood, this left the genetic counselor certain of what should happen next: Claudia needed to be tested for mutations in *DICER1*, which can predispose to specific types of benign and malignant tumors. Claudia's test came back positive, and so, not surprisingly, did Seymour's.

Yet a seemingly inherited mutation in a child with cancer isn't always accompanied by an arresting family history. In November 2015, St. Jude reported on a cohort of more than 1100 children whose tumor and noncancerous cell DNA were sequenced. In DNA from their normal cells, 8.5% of children had potentially harmful mutations in cancer genes. Yet among those children, only 40% of those for whom a family history was available had relatives with cancer. How was that possible?

One explanation is that the mutation might be new, having popped up spontaneously when the child was conceived. Schiffman suggests another: Doctors "do a lousy, lousy job of collecting family history" and may be deaf to alarm bells in an extended family. A "ringing message" of the new wave of sequencing, Schiffman says, is that every single child with cancer needs a detailed history taken at diagnosis.

But family history is also fluid: Over time, a sunny family history can flip. "This just happened to one of my own patients," says Malkin of Toronto's Hospital for Sick Children. He treated a teenager with adrenal carcinoma, a disease often caused in children by inherited mutations. When genetic testing came back clean, doctors concluded this youngster was an exception. Then, 4 years later, the boy's mother developed breast cancer. "She's young enough that one has to believe there's a connection," Malkin says. DNA diggers were likely looking in the wrong place.

Ultimately, even an impeccable family history doesn't rule out cancer mutations lurking in the genome and later causing disease. Families are far smaller than they used to be, says Sharon Plon, a medical geneticist at Texas Children's, which means that a mutation with, say, a 30% chance of causing cancer might never have a chance to bare its teeth twice in a single family.

**ABOUT 3 YEARS AGO**, Plon teamed up with Parsons, the brain cancer physician, and they began reaching out to Texas Children's families 2 months after their children were diagnosed with a solid tumor. As part of a research study called BASIC3, each child was offered sequencing of both tumor and

nontumor DNA. Then Plon and Parsons went a step further: In addition to studying the patients and families, they enrolled their doctors, probing how the oncologists felt about returning sometimes confusing or difficult genetic results.

The doctors and genetic counselors are sharing not only cancer-predisposing mutations that might or might not have fueled that child's illness. They are also offering up genetic findings unrelated to cancer if treatment or monitoring can help—for example, mutations that cause the life-threatening heart condition long QT syndrome or familial hypercholesterolemia. (They do not return results when there is no way to act on them. No 8-year-old is finding out whether she's at elevated risk of Alzheimer's disease, for example.) Families also learn whether their child has so-called "variants of unknown significance" in cancer genes, as most do.

Parsons was struck by how easy it was to recruit the participants, who now include roughly 250 patients. "Our families will agree to just about anything," he says. That sometimes worries him, as he wants to be sure these parents, already facing a devastating blow, grasp what might come next for the affected son or daughter and the rest of the family. The findings can mean recommending genetic testing on siblings, full-body MRIs for parents, or a lifetime of surveillance of the ailing child, because those who survive one cancer may have a high chance of developing another.

Yet parents whose child has cancer may view genetic information through an altogether different lens than would a healthy—and potentially wary—adult. "Yeah, sure, you're going to have some anxiety, and yeah, sure, you're going to learn some things that you may not really want to know," one parent shared, in a paper the group published in September 2015 on the ethics of whole-exome sequencing in these children. "But I just—I couldn't live with myself if we were in the same situation in 2 years and I had a chance to at least know about it."

In the disorienting time after Claudia's diagnosis of rhabdomyosarcoma, Seymour's emotions mirrored those of the families in the Houston study. With her rising third-grader enduring major surgery and months of chemotherapy, "my counselor would tell you I literally ran from her," she says. Genetic counseling, with any abstract future worries it might highlight, sank to the bottom of her priority list.

But as weeks passed, Seymour's mindset evolved. Claudia completed treatment in November 2015. She continues to recuperate at home, where she is a "craft maniac," making soaps, snow globes, lip balm, and lava lamps thanks to YouTube instructional videos. Seymour, meanwhile, says she decided to "stop thinking of it as, 'There's nothing they can do.'" She is waiting on *DICER1* results for her two younger children, 6- and 1-year-old boys. If one or both test positive, St. Jude would recommend regular screening, including chest x-rays and kidney and thyroid ultrasounds that might catch tumors early.

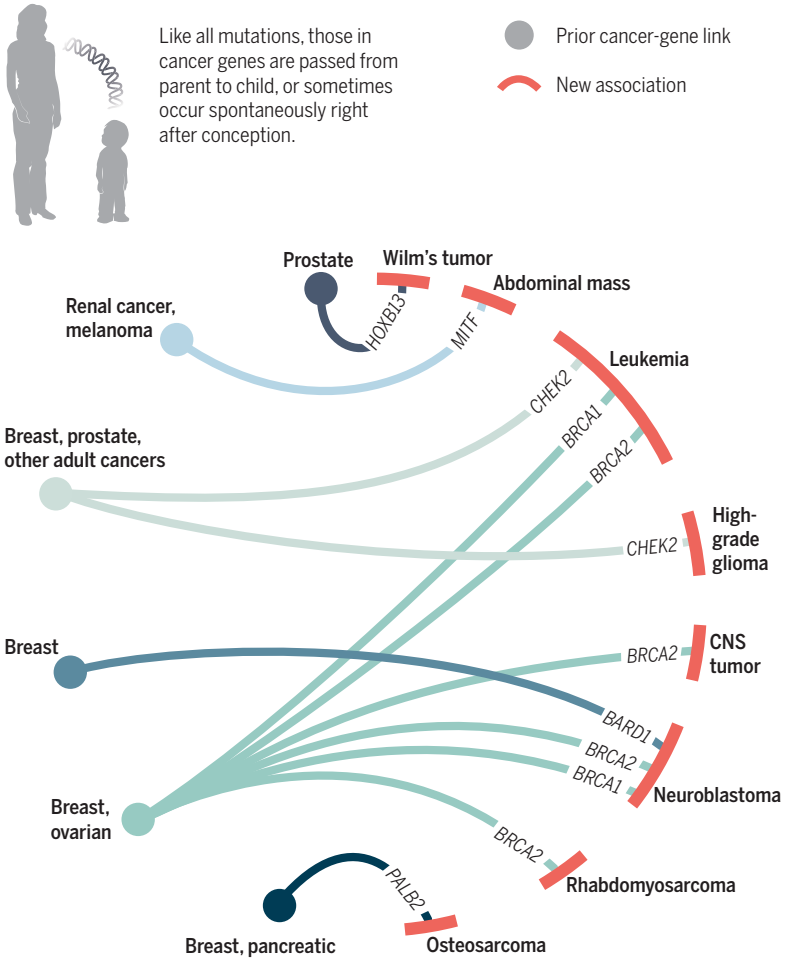
Right now many inherited mutations in pediatric cancer patients are identified because of a suspicious family history like Claudia's, or as part of broader research efforts like Plon's. Plon, for one, isn't quite ready to go beyond that and offer full DNA sequencing of noncancerous tissue to every child with cancer who walks into her hospital, but other centers are moving in that direction to varying degrees. They include St. Jude, UM, The Hospital for Sick Children, and Intermountain Primary Children's Hospital in Salt Lake City, where Schiffman, the lymphoma survivor, works. He had his own genome sequenced as part of a research study; many variants of unknown significance cropped up, but nothing to suggest known cancer predisposition for his three children or himself.

Oncologists hope that at least some new findings about the inborn risks of cancer will eventually lead to better treatments: Already one drug is approved for women with advanced ovarian cancer who carry *BRCA* mutations. To try to speed the science along, an NCI-funded study called Pediatric MATCH plans to start rolling out across more than 190 U.S. centers. It will offer tumor and nontumor DNA sequencing to children with cancer, aiming to identify molecularly guided therapies, and potentially families who might benefit from genetic testing and surveillance.

Right now, however, the new biology can generate electrifying connections and inform a family, but without saving the life that helped spawn that knowledge. The link Walsh discovered between a *P53* mutation and his young patient's leukemia advanced understanding of that disease but didn't help the child. The boy died when he was 11 years old. His two siblings tested negative for mutations in *P53*. ■

## Crisscross biology

Genetic sequencing is showing that the normal cells of some kids with cancer harbor gene mutations with known links to different adult cancers. Scientists are trying to understand whether these gene changes could explain the kids' cancers or are largely or entirely unrelated to a child's disease.




GRAPHIC: V. ALTOUNIAN/*SCIENCE*; (DATA) J. ZHANG ET AL., *THE NEW ENGLAND JOURNAL OF MEDICINE* (18 NOVEMBER 2015); R. J. MODY ET AL., *JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION* **314**, 913 (1 SEPTEMBER 2015); SHARON PLON/BAYLOR COLLEGE OF MEDICINE/TEXAS CHILDREN'S HOSPITAL