Novel NextGen sequencing test developed for retinoblastoma

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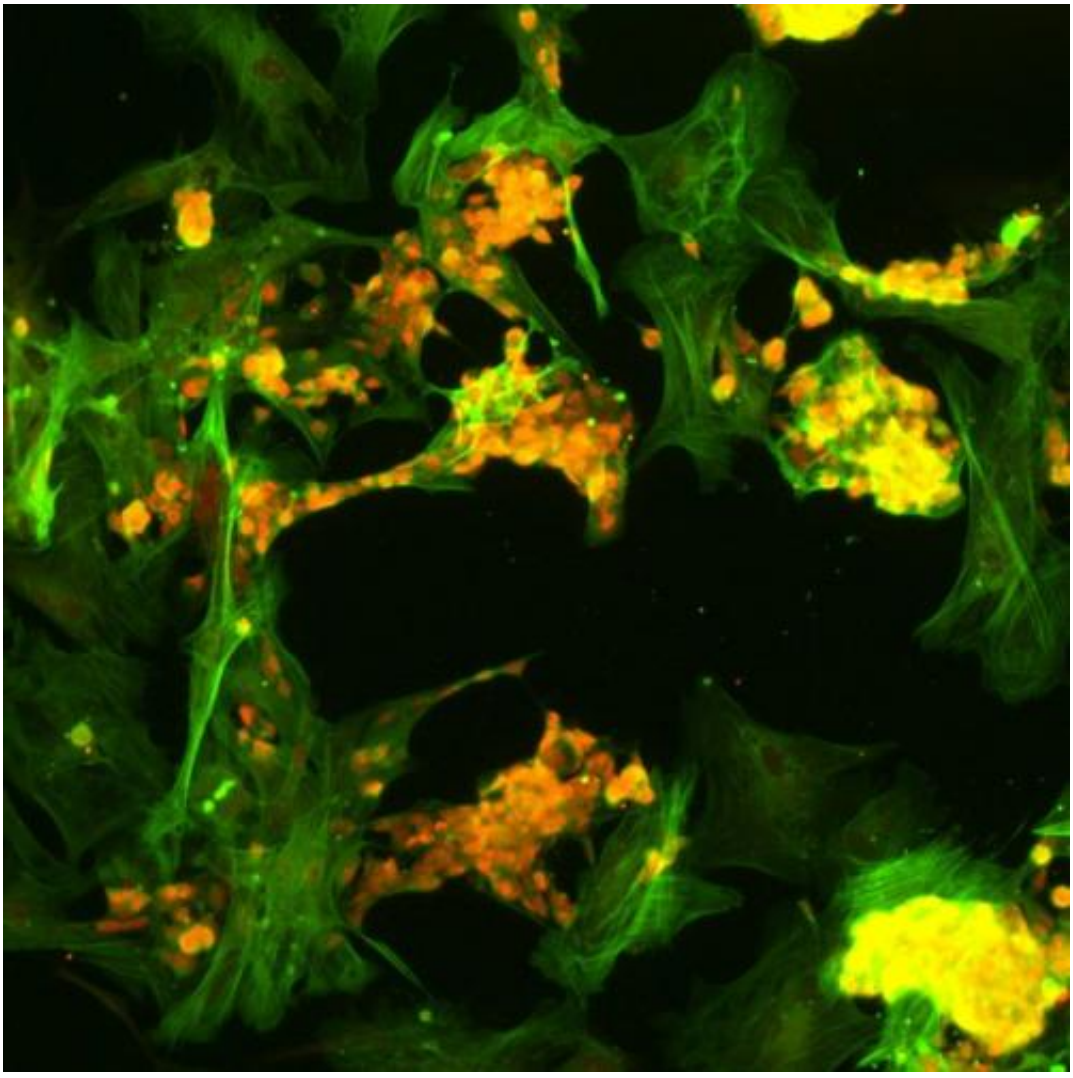

This shows retinoblastoma tumor cells. Credit: Tom Lee, MD

Researchers at Children's Hospital Los Angeles (CHLA) have developed a unique next-generation sequencing test for the gene linked to retinoblastoma. The new approach is both more comprehensive and can be conducted in less time than existing tests – enabling early detection of the cancer's genetic cause, which is critical for optimal treatment outcomes.

The test was developed to serve the needs of the Retinoblastoma program at CHLA's Vision Center, one of the largest clinical programs in the U.S., caring for approximately 20% of the nation's retinoblastoma cases and seeing as many as 50 new patients each year. Retinoblastoma – a malignant cancer of early childhood that arises from immature retinal cells in one or both eyes – can start growing at any time before birth up until about seven years of age. Among infants and children, common signs of this cancer include having a white "glow" or "glint" in the pupil of one or both eyes, the presence of a white pupil in a color photo, or crossed or misaligned eyes.

Early detection is vital because, when left untreated, retinoblastoma can result in blindness or death. In germline cases, the disease causing mutation is present in cells throughout the body resulting in tumors in both eyes, as well as in other locations throughout the body. Therefore, early detection and accurate diagnosis are critical for determining the best course of treatment.

A team of physicians and scientists from the Department of Pathology and Laboratory Medicine's Center for Personalized Medicine, working with clinicians and surgeons from the Vision Center, developed the CHLA retinoblastoma next generation (RB1 NextGen) sequencing panel based on deep DNA sequencing technology, using novel technical and bioinformatics methodology to sequence the entire RB1 gene.

Conventional genetic testing for retinoblastoma typically involves

sequencing of specific hot spots within the gene where there are known to be numerous mutations. When CHLA researchers set out to develop their own laboratory-derived test, they took a very different approach in order to sequence the entire gene from end to end.

"Typically, we have used NextGen sequencing capability to sequence many genes at a relatively low depth of coverage" explained Alexander Judkins, MD, FRCP, head of the Department of Pathology and Laboratory Medicine. "Our team took a very different approach by deciding to look at a high depth of coverage of a single gene, sequencing through the entire gene, which is technically very difficult to do."

With the existing NextGen sequencing approach for retinoblastoma, there are regions of genes that do not get examined at all, potentially allowing significant data to be missed, added Timothy Triche, MD, PhD, Director of the Center for Personalized Medicine at CHLA. "New data tells us that the non-coding DNA is extremely important. And one of the strengths of our approach is in examining those non-coding areas."

By sequencing the whole gene, researchers are able to see more frequent occurrences of germline mutations of the RB gene. Because germline mutations can be passed on, this knowledge can better inform the correct treatment approach for patients when these mutations are identified.

"We used to think that if a child had a tumor in one eye and not in the other eye, it was a result of a somatic mutation. And because somatic carriers don't pass on mutated genes, we believed removal of the tumor stopped the cancer," says Tom Lee, MD, Director of the Vision Center at CHLA. "But now we know that there is a percentage – on the order of 10 to 15% of those children who had an apparent somatic mutation – who are actually carrying a germline mutation, putting them at risk of developing subsequent tumors during adolescence or early adulthood. "

Lee said that the ability to determine the existence of RB1 germline mutations early has significant implications – not only for the prognosis of a new patient with retinoblastoma, but for siblings who could also be at risk and eventually for a patient's own children, if a germline mutation is detected in a retinoblastoma survivor that could be passed on to future generations.

With a legacy of treating hundreds of children with retinoblastoma, CHLA now has the ability to also offer this unique and comprehensive screening test to survivors of the disease or their family members, as well as to patients at other cancer centers. "We believe that siblings and children of carriers of the germline should be tested immediately after birth because by detecting the gene mutations early can save a patient's vision," says Lee.

Approximately two-thirds of retinoblastoma patients at CHLA have unilateral cases of RB, or cancer in just one eye. While only 15% of cases will test positive for a germline mutation, physicians must treat all patients as though the cancer could potentially be bilaterial, or present in both eyes, a condition commonly associated with having a germline mutation. "Historically, genetic testing can take up to three or four months—which is a very long time when a child's health is at stake," said Jonathan Kim, MD, director of the Retinoblastoma Program at CHLA. "Doing our tests faster and on site has the potential to significantly improve treatments outcomes for all of our patients."

"This test enables our researchers to look at the RB1 gene more comprehensively," says Judkins. The collection of new genomic data on RB1 will hopefully enable basic researchers to translate this material into biologically meaningful insights, leading to earlier detection and new treatments for both primary and recurrent disease."

Provided by Children's Hospital Los Angeles