The power of precision genomics to understand unique causes of disease in individual patients

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A UC San Francisco-led research team has identified the rare genetic mutation responsible for a unique case of severe combined immunodeficiency (SCID), a deadly immune system disorder also known as "boy in the bubble" disease. In addition to defining the latest

of more than two dozen known genetic causes of SCID, the study—published online November 30, 2016 in the *New England Journal of Medicine*—revealed an unexpected role for the mutated gene in the normal processes of immune system development.

"We're entering a new era of genomic medicine," said Jennifer Puck, MD, UCSF professor of immunology and pediatrics, a pediatric immunologist at UCSF Health and senior author of the new study. "Our technology has progressed to the point that we can learn a great deal about a disease, and even learn important new facts about normal biology, from just a single patient. In this case we were able to unearth the potentially unique underlying genetic cause of one patient's disease and come away with brand new understanding of how the immune system develops."

The patient featured in the new study was identified through a population-based neonatal screening approach for SCID that Puck developed in 2005, and which is now widely performed. (Since it was introduced in California in 2010, Puck's screening method has increased the survival rate of infants with SCID to 94 percent, and by 2017 will be used in 47 US states.) The screening indicated a severely compromised immune system, leaving the patient open to a likely fatal series of infections. However, UCSF doctors performed a bone-marrow transplant, the standard of care for SCID, which provided the infant with a fully functional immune system.

In addition to SCID, however, the infant was also born with a constellation of abnormal features including craniofacial deformities, loose skin, excess body hair, and neurological abnormalities, which suggested that a single rare genetic defect could underlie the patient's disease. In part to determine whether the infant's parents were carriers of a genetic mutation that could be passed on to future children, Puck's group set out to scan the genomes of both infant and parents for

mutations that could be responsible for the disease. Working with the lab of computational biologist Steven Brenner, PhD, of UC Berkeley and with researchers at Tata Consultancy Services, the team used next-generation exome sequencing to identify a single mutation present in the infant but not the parents—referred to as a de novo mutation—in the BCL11B gene, which had previously been associated only with lymphatic cancer.

"What's remarkable is that not only was this a gene that had never been associated with SCID before, which required more advanced genomics techniques to discover," said Brenner, "but also that unlike every other known SCID gene, you only need one copy of this mutation to disrupt multiple aspects of development."

In order to understand the biological effects of the patient's mutation, the researchers collaborated with the team of David Wiest, PhD, of the Fox Chase Cancer Center in Philadelphia, to introduce the patient's mutated form of BCL11B into zebrafish, whose immune systems are similar to those of humans. They found that the mutated form of BCL11B produced abnormalities in the zebrafish that mimicked those observed in the patient, including not only a disabled immune system but also similar craniofacial abnormalities. Blocking the mutated gene and replacing it with the normal human gene in embryonic zebrafish reversed all these symptoms, strongly suggesting that abnormal BCL11B was the cause of the symptoms seen in both zebrafish and the human patient.

The normal BCL11B protein binds to DNA at sites across the genome to activate a wide variety of developmental genes in a precisely orchestrated sequence. Experiments revealed that the BCL11B gene mutation identified in the new study disrupts this protein's ability to bind to DNA, thereby resulting in the wide array of immunological, neurological, and craniofacial disruptions seen in both the human patient and in zebrafish.

"Mutations do arise on the way from the joining of sperm and eggs to producing a new person," Puck said. "Everyone has such new mutations, but usually they are silent passengers that don't do any harm. In this case, however, a mutation in BCL11B turned the protein it produces into a monkey wrench that disrupted many different systems in the body."

Because zebrafish embryos are transparent, the researchers were able to observe that one key effect of disrupting BCL11B was to block the ability of immature bone marrow stem cells to successfully migrate into the thymus, where these cells are normally "educated" to become mature T lymphocytes, often called 'T cells', which are essential for combating infection and are almost completely lacking in patients with SCID.

Further experiments in the lab in which the researchers introduced the BCL11B mutation into normal human bone marrow stem cells and compared them with diseased cells obtained from the patient confirmed that this infant's mutation impaired its T cells' ability to migrate and mature.

According to Puck, the findings illustrate the power of deeply studying rare diseases in individual patients: "We may never get another patient just like this one," she said. "But as a result of studying this one case we were able to learn so much about a critical gene in a critical pathway that hadn't been appreciated before."

More information: Divya Punwani et al. Multisystem Anomalies in Severe Combined Immunodeficiency with Mutant, *New England Journal of Medicine* (2016). DOI: 10.1056/NEJMoa1509164

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