Scientists find gene variants causing NK cell deficiency, solving 12 year-long mystery for a family

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An international team of scientists has solved a medical mystery that has affected a family for more than 40 years. The condition made most of the children in the family susceptible to severe viral infections, Epstein-Barr virus in particular. Two of the four siblings died due to the infections, one was unaffected by the condition and one has survived repeated infections. Discovering the cause of the condition has brought closure to the family, personal satisfaction to the researchers and a better understanding of the clinical manifestations of deficiencies in natural killer (NK) cells, the first responders to viral infections. The results appear in The *Journal of Clinical Investigation*.

"The medical history of this family was first reported in 1982," said Dr. Emily Mace, assistant professor in pediatrics-allergy and immunology at Baylor College of Medicine and first author of the paper. "The 1982 paper describes the first example of an NK cell deficiency, but it was years later in 2004, when research continued to try to discover the cause of this deficiency."

In 2004, the surviving affected sibling of this family entered the Adult Immunology Clinic at Penn Medicine. He was ill and requesting assistance from the staff. As he was holding the 1982 scientific paper in his hand, he stated that he was the person mentioned in the paper; that he had no NK cells in his body.

When the patient went to the Clinic, Dr. Jordan S. Orange, senior author of this work and currently professor and section head for immunology, allergy and rheumatology in pediatrics at Baylor College of Medicine and director of the Center for Human Immunobiology at Texas Children's Hospital, was an assistant professor of Pediatrics and Immunology at Penn in 2004. .

"I was called at the Adult Immunology Clinic as the staff knew that I was very interested in the field of NK cell deficiency and its clinical relevance. The one surviving member of this affected family was there. The staff asked me if I would be interested in seeing him," Orange recalls. "I spilled my coffee on my lap and ran to the Clinic. I wanted to explore the possibility of working with this patient to learn as much as possible about his condition. I met him and soon began working with the family. At the time, I was beginning to establish my own lab; I worked with the patient directly."

This fortuitous encounter marked the beginning of a 12 year scientific investigation. As Mace and Orange later joined Baylor and continued this work, research tools they needed to answer their questions had improved. The researchers established collaborations that gave them access to the techniques as well as to the experience of many other researchers.

"At Baylor, we started a collaboration with other groups, including the lab of Dr. James R. Luspki Cullen Professor of Molecular and Human Genetics and director of the Center for Mendelian Genomics at Baylor," said Mace. "Our research team was able to do whole exome sequencing – sequencing all the genes expressed in the genome – in a number of families that had this clearly familial NK deficiencies. However, the family in the 1982 paper was one of the first for which we really wanted to solve the case."

Solving the mystery

"Over the years, we worked with the remaining family members," said Mace. "We studied the genes of the patient, the mother, the unaffected sibling and the patient's three children."

"We decided to pursue this research although at the time (2004) we were not funded to do so," said Orange. "For the last year now, our research is funded under a National Institutes of Health grant in The Genetic and Mechanistic Basis of Human NK Cell Deficiencies, which has further accelerated our path to the finish line."

The researchers sequenced the genetic material of the family members and were able to pinpoint two rare variants of the gene IRF8, called P224L and A201V, as the cause of their condition. Variant A201V had not been reported before.

"We determined who has each variant of the gene," said Mace.

The mother carries one of the variants and a normal gene. The unaffected sibling carries the other variant and a normal gene. Neither of them is affected by the condition because the normal gene allele compensates for the disease-causing variant. The surviving sibling carries both variants, P224L and A201V.

"We also found that the patient's children were carriers but unaffected," said Mace. "This news brought much relief to the family. One of the main lessons we learned is that to have NK cell deficiency as a result of IRF8 mutations, both copies of the gene have to be mutated into defective copies."

The researchers also studied the effect of the disease-causing variants on NK cells. They discovered that the individuals carrying two defective

IRF8 variants have fewer mature NK cells than those with the normal gene, and these fewer cells do not work properly. These conditions result in a lack of an effective response against viral infections.

"This paper underscores the importance of NK cells in the control of viral infection," said Mace. "This is one of the purest cases of a loss of NK cells being responsible for fatal viral infections. We have studied other cases with other families with NK deficiencies and found that the clinical hallmark is severe viral infection, particularly with herpes virus, including Epstein-Barr virus."

Beyond research

"Visiting the family's home was one of the greatest rewards of being a physician-scientists that I have experienced," said Orange. "I sat with this family and went through the story of what they suffered, and thanks to our persistent research I was able to provide some answers. It was probably one of the highlights of my career."

"The thought of being able to put this paper into this family's hands was a driving force in keeping the work going," said Mace. "It's tremendously rewarding that we have been able to provide solace to this family."

More information: Emily M. Mace et al. Biallelic mutations in IRF8 impair human NK cell maturation and function, *Journal of Clinical Investigation* (2016). DOI: 10.1172/JCI86276

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