A multinational collaboration has managed to unravel a particular gene’s complicity in a particular kind of leukemia, a small but important step in understanding a blood disease that afflicts more than 50,000 new patients a year in the United States.

It started with a couple of University of Colorado Cancer Center researchers visiting Detroit.

In March 2013, Jorge Di Paola, MD, and Leila Noetzli, a PhD student with the CU School of Medicine’s Human Medical Genetics and Genomics Program, went to Motown looking for blood. Di Paola is a School of Medicine hematologist specializing in blood platelets – thrombocytes, technically, which are best known for stopping bleeding. He sees patients at University of Colorado Hospital and Children’s Hospital Colorado.

The reason for the trip? A couple of Wayne State University professors and pediatric hematologists with Children’s Hospital of Michigan, Madhvi Rajpurkar, MD, and Michael Callaghan, MD, had come across a family with interesting blood. They all had big red blood cells and low platelet counts, which affected clotting, meaning they bled easily. But they bled more than many with similarly low – but not extremely low – platelet counts. And also, two of the family members, an aunt and her niece, had acute lymphoblastic leukemia (ALL), a rare form of blood cancer.

Di Paola and Noetzli sampled 14 members of the family – four sisters and their 10 children, adding to Di Paola’s bank of more than 2,000 samples (half being from a single, extended Amish family).

“Our lab always works with families to try to understand the genetic origins of blood disease,” Di Paola explained.

Family ties. Back in Colorado, Di Paola met with Ken Jones, PhD, co-director of the CU Cancer Center’s Biostatistics and Bioinformatics Shared Resource. Jones, who leads the bioinformatics group, called in Katherine Gowan, a professional research assistant with the lab. Relationships between subjects matter in biostatistics. Jones and Gowan said sequencing eight of the family members would suffice. Having four sisters and ten cousins to choose from was a boon, Jones said.

“Two cousins share 6 percent of their genes by random chance,” he said. “If you have three, they only share six percent of six percent.”

Meaning, he said, if there’s a genetic disorder that’s dominant in a family, more cousins means a better chance that common genes are causing the problem.

It took a month to do the sequencing (CU’s DNA Sequencing and Analysis Core did that), during which time Gowan readied the bioinformatics software. The sequencing brought back a total of 160,000 genes (each human has approximately 20,000 genes) – all

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part of the eight subjects’ exomes, the roughly 2 percent of human DNA known to encode proteins.

Match game. Typically, even a sample set as good as Di Paola’s and Noetzli’s would yield just 10 to 200 shared gene variants, which Gowan described merely as “a list of candidates.”

“It’s things that all the people in a family share just by chance, like ‘All these people have blue eyes,’ and it doesn’t necessarily have anything to do with your disease,” she said.

She ran the gigabytes of data through exome sequencing software that didn’t exist until 2011. The output: a single shared gene. That happens, Gowan said, “not ever.”

“My initial reaction was utter shock that there was only one line,” she said.

Her second impulse was to look up the gene’s function.

“If it had said it’s only expressed in our toenails in a fetal stage, then I would have been really depressed,” she said.

But it was blood-related. She called Di Paola.

“I was like, ‘What’s ETV6?’” Di Paola recalled.

The platelet guru hadn’t heard of the gene because, as Noetzli explained, “It has never been described in platelet disorders in humans – only in leukemia, and it is also known by a different name, TEL.”

Christopher Porter, MD, a CU School of Medicine leukemia expert who, like Di Paola, sees patients at UCH and Children’s Colorado, occupies the office next door to Di Paola’s. Di Paola had kept Porter abreast of the Detroit work. Porter was most interested in this family’s potential to help isolate the genetic underpinnings of ALL.

ETV6 mutations are behind most childhood leukemias, Porter said. But those mutations are usually somatic – that is, the result of normal genes somehow going bad, often under pressure from other mutated genes. Having a family with a germline, or inherited, mutation could help isolate the impact of the ETV6 mutation.

Porter was, as Di Paola put it, “all excited.” He recruited a junior faculty member from Children’s Colorado, also a leukemia expert, to help.

Widening net. At that point, the work took on new collaborators far and wide. Porter recruited CU School of Medicine and Children’s Hospital Colorado leukemia expert Alisa Lee-Sherick, MD. Di Paola contacted colleagues in Italy, who in turn touched base with others in the Czech Republic. The Europeans sent samples from two families, one Italian, one Czech, all with the ETV6 mutations.

For more than a year, Noetzli worked on cell cultures to see how the ETV6 mutation affected the platelet-producing megakaryocyte cells in blood cell-producing human bone marrow. The protein ETV6 is a transcription factor that appears to be responsible for genetic alterations, many of which remain a mystery, Noetzli said. But it was clear that ETV6 was affecting cell function. Cell biologist Walter Kahr, MD, PhD, of the University of Toronto demonstrated the effect vividly (see graphic). Using immunofluorescence microscopy, he showed that mutated ETV6 proteins couldn’t get from the cell’s cytoplasm into the nucleus, where they belonged.

Their work was published in the prestigious Nature Genetics on March 25; at about the same time, a University of Washington team led by Akiko Shimamura, PhD, published similar, independent findings, strengthening the case for the role of ETV6 mutations in leukemia.
The case is far from open-and-shut. Di Paola said hundreds of genes may be involved in leukemia. Porter’s team is now studying B-cells, a type of white blood cell, to identify other genes that may be sucked into ETV6’s cancerous cascade.

Di Paola and Noetzli are working with University of Utah researchers to develop a mouse model with the ETV6 mutation. Di Paola and Noetzli, too, are looking at downstream effects of ETV6, but on platelet production and behavior.

For Di Paola, the discovery is another example of how scientific progress increasingly depends on working across national and professional boundaries.

“If you don’t share these ideas, you really get boxed into single-minded science that doesn’t work,” Di Paola said. “This is how collaborative science is now. It’s almost impossible to do it yourself.”