When sequencing fails to pinpoint a rare disease

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Routine sequencing has given unprecedented insight into the genetics of rare diseases, but genomics fails to diagnose up to half of patients who are tested. That's the problem German scientists tackled in a recent study in the journal *Molecular & Cellular Proteomics*. Using samples from patients in four countries, and a novel database on the neutrophil proteome, they were able to make genetic diagnoses for two children with severe congenital neutropenia whom typical sequencing had failed.

"There are very few examples of people who use multiple '-omics' to investigate rare diseases ... (but) I think this is the future of personalized medicine," said senior author Christoph Klein, a physician and the director of the Children's Hospital of the University of Munich.

The patients' disease affects their neutrophils, white blood cells packed with toxic proteins to deploy against bacteria. When neutrophil development is disrupted, which Klein estimates happens to 1 in 200,000 newborns, every bacterial or fungal infection can become a life-threatening medical emergency.

Neutrophils are fragile, which makes them difficult to study. Postdoctoral researcher Sebastian Hesse developed a protocol to collect proteins from healthy neutrophils. Then scientists led by Piotr Grabowski in Juri Rappsilber's proteomics lab at the Technical University of Berlin used those healthy cells to establish a baseline neutrophil proteome.

Then, Hesse collected neutrophils from 16 patients with congenital neutropenia. Some of them lived in Germany; to reach others, he had to travel as far as Turkey and Iran. Mass spectrometrists repeated the same proteomic assay to compare patients' neutrophils to volunteers'.

The team used abnormal protein profiles to diagnose two patients with inconclusive exome sequencing results. In one child's case, a pseudogene made it difficult to identify mutations in the protein-coding gene; in the second, incomplete coverage by exome sequencing had missed a key point mutation. Data on protein abundance in each patient led the researchers to run secondary genetic analyses that proved conclusive.

Both of these mutations are known causes of neutropenia. "This highlights (that) even if you have highly controlled pipelines for genetic studies, there's always a risk that you are not 100 percent correct," said Klein. In a forthcoming paper the team will report on novel genetic causes for neutropenia found using the proteogenomic technique.

Combined proteomic and genomic screening is not yet practical for every patient. "But, if you look at the machines that are currently being developed, I think there will be huge potential for proteome analysis at a very low cost down the road," Klein said.

More information: Piotr Grabowski et al, Proteome analysis of human neutrophil granulocytes from patients with monogenic disease using data-independent acquisition, *Molecular & Cellular Proteomics* (2019). DOI: 10.1074/mcp.RA118.001141

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