

Molecular Pathology Selected Abstracts, 7/16

Editors: Donna E. Hansel, MD, PhD, chief, Division of Anatomic Pathology, and professor, Department of Pathology, University of California, San Diego; John A. Thorson, MD, PhD, associate professor of pathology, director of the Clinical Genomics Laboratory, Center for Advanced Laboratory Medicine, UCSD; Sarah S. Murray, PhD, professor, Department of Pathology, and director of genomic technologies, Center for Advanced Laboratory Medicine, UCSD; and James Solomon, MD, PhD, resident, Department of Pathology, UCSD.

Using single nucleotide polymorphism arrays in acute lymphoblastic leukemia

In patients with acute lymphoblastic leukemia/lymphoma, the identification of chromosomal abnormalities at the time of diagnosis is important for risk classification. The current standard of care includes karyotype and FISH analysis to identify recurrent cytogenetic abnormalities. However, many hematologic malignancies will not demonstrate an abnormality using these techniques because karyotyping requires microscopic examination of cells in metaphase and FISH only probes specific genetic sequences. Whole-genome single nucleotide polymorphism (SNP) arrays allow for identification of both copy number alterations and copy neutral loss of heterozygosity at a submicroscopic level across the entire genome, providing a much higher resolution analysis of the chromosomal abnormalities associated with hematologic malignancies. The authors conducted a study in which an SNP array—in which extracted genomic DNA is analyzed with approximately 2.7 million probes—was used in 60 consecutive patients. They determined copy number and genotype of each of the probed areas and compared data to databases of known common copy number variations seen in healthy control subjects. The authors reported gains, losses, and areas of loss of heterozygosity. The SNP array data were integrated with karyotype and FISH analysis to characterize the chromosomal abnormalities. Of the 60 cases, abnormalities in karyotype and FISH were seen in 81 percent and 78 percent of cases, respectively. However, abnormal SNP array results were observed in all 60 acute lymphoblastic leukemia/lymphoma cases, although clinically relevant abnormalities that had prognostic or therapeutic implications were seen only in 34 cases. More importantly, however, combining the SNP array results with the karyotype and FISH analysis increased the detection rate for clinically relevant abnormalities from 56 percent to 75 percent of the leukemia/lymphoma cases. One interesting application is in detecting IKZF1 deletions, which are associated with poor response to induction therapy and overall poor prognosis. Current recommendations are to assess for deletions in IKZF1 and, if positive, perform additional ancillary testing to find targetable tyrosine kinase mutations. In the study, deletion of the IKZF1 gene was seen in 30 percent of cases, with deletions varying in size from 0.03 to 6.2 Mb. While other assays can assess for deletions in IKZF1, many of them are specific for the gene, whereas SNP microarray appears to be equally sensitive while being able to detect other submicroscopic chromosomal alterations and areas of loss of heterozygosity. A caveat is that SNP microarrays are not effective at detecting balanced translocations. Fifteen cases in the current study had balanced translocations seen by karyotype or FISH, or both, 13 of which could not be detected by the SNP microarrays. The authors concluded, however, that when used in conjunction with karyotype and FISH analysis, whole-genome SNP microarrays greatly increase the ability to detect chromosomal alterations that may be clinically relevant.

Wang Y, Miller S, Roulston D, et al. Genome-wide single-nucleotide polymorphism array analysis improves prognostication of acute lymphoblastic leukemia/lymphoma. *J Mol Diagn.* 2016;18. doi:10.1016/j.moldx.2016.03.004.

Correspondence: Dr. Lina Shao at linashao@med.umich.edu

Role of exome sequencing in managing neurometabolic disorders

The discovery of genes associated with rare mendelian diseases has been revolutionized by next-generation

sequencing technologies. An estimated three percent of the population is affected by an unexplained intellectual developmental disorder. It has been suggested that whole-exome sequencing be used to find a causative diagnosis so that appropriate genetic counseling and medical support can be provided. In some cases, especially for the neurometabolic disorders, there may even be disease-modifying treatments. The authors performed whole-exome sequencing on 41 patients who had intellectual developmental disorders and an unexplained phenotype, which was defined as abnormalities in urine, blood, or cerebrospinal fluid metabolites, abnormal biochemistry studies, abnormal histology findings, such as storage vacuoles, or abnormal clinical history, physical exam, or imaging findings. They established a genetic diagnosis in 28 of 41 (68 percent) of the patients. The study also identified 58 diagnostic variants in 42 genes, including the discovery of two novel genes that are newly implicated in human disease and are defined as novel because they had damaging variants in at least two unrelated patients with phenotypic overlap. In addition, nine candidate genes, 22 genes with newly identified phenotypes, and nine genes with expected phenotypes were found. The study further described the two novel genes with illustrative cases. The first case was caused by a homozygous missense variant in CA5A, encoding mitochondrial carbonic anhydrase VI. Deficiency in this enzyme causes neonatal hyperammonemia, hyperlactemia, and hypoglycemia, and can be treated with carnitine. The second case was caused by compound heterozygous variants in NANS, encoding N-acetylneuraminic acid phosphate synthase. To confirm the diagnosis, the substrate of the enzyme N-acetylated mannosamine was detected in the patient's urine, plasma, and cerebrospinal fluid. In addition to the discovery of the two novel genes, new clinical phenotypes were seen in some patients who had variants in genes previously reported to cause other conditions or syndromes. The study also found a few patients that had variants in multiple genes known to cause a few separate syndromes, resulting in an overlapping complex phenotype. Perhaps the most promising finding of the study was that the genetic diagnosis was able to affect clinical treatment of 18 of the 41 (44 percent) patients. The authors concluded that the application of germline whole-exome sequencing to patients with neurometabolic disorders may lead to increased understanding of the biochemical pathogenesis of these disorders and afford clinicians the opportunity to treat with nutritional manipulation or other targeted treatments.

Tarailo-Graovac M, Shyr C, Ross CJ, et al. Exome sequencing and the management of neurometabolic disorders. *N Engl J Med*. 2016; 374:2246-2255.

Correspondence: Dr. C. D. van Karnebeek at cvankarnebeek@cw.bc.ca