Molecular pathology selected abstracts

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Exome sequencing in infantile-onset pharmacoresistant epilepsy

December 2022—Infantile-onset epilepsy has a variety of underlying etiologies, including brain injury, metabolic disorders, and genetic factors. Refractory epileptic conditions in infants can result in developmental delays, poor quality of life, and increased mortality rates. Timely diagnosis and management is challenging given the degree of clinical and genetic heterogeneity inherent in this condition. Next-generation sequencing (NGS) methodologies have had a significant impact on the ability to identify causative genes. However, few studies have addressed when during the course of patient diagnosis and management NGS should be performed. The authors investigated the diagnostic and therapeutic utility of exome sequencing in a cohort of 103 unrelated patients with infantile-onset pharmacoresistant epilepsy. Cases were considered to be solved if pathogenic or likely pathogenic variants in genes consistent with the phenotype were identified and zygosity matched the inheritance pattern. Cases were considered to be partially solved if they harbored one heterozygous pathogenic or likely pathogenic variant in a gene associated with recessive inheritance. The initial exome sequencing analysis resulted in 61 (59 percent) cases being solved and two (two percent) being partially solved. Re-analysis of exome- sequencing data in the unsolved cases led to three more cases being solved. Through initial and repeat analysis, exome sequencing solved 62 percent (64 of 103) of cases. Both of the partially solved cases were further analyzed using short-read and long-read genome sequencing, or both, which identified additional novel pathogenic alterations, solving both cases. Forty-three percent (44 cases) of the study cohort harbored causative variants that had therapeutic implications. Eight patients had neurometabolic disorders for which treatment prevented seizures. Exome sequencing most commonly identified disease-causing variants in *SCN1A* (13 percent; 13 of 103) but also in *ALDH7A1, PNPO, BTD, SCN2A, SCN8A, ATP1A3, KCNA2, KCNT1, KCNQ2,* and *PDHA1*. The most common treatable disorder in the cohort was autosomal-recessive pyridoxine-dependent epilepsy, with six patients harboring pathogenic or likely pathogenic variants in the *ALDH7A1* gene. Seizure semiologies, electroencephalogram and neuroimaging findings, and number of epileptic drugs used before testing showed no significant association with variant identification. This suggested that such clinical information may not be useful in predicting the diagnostic yield of genetic testing in patients with infantile-onset pharmacoresistant epilepsy. Notably, the high diagnostic yield of exome sequencing in this study was influenced by several factors, including thorough exclusion of acquired causes of seizure prior to testing and use of trio (the proband and that person's parents) NGS analysis in the majority of cases. This study supports the early use of genetic testing in patients with infantile-onset pharmacoresistant epilepsy to facilitate timely diagnosis and improve patient management.

Boonsimma P, Ittiwut C, Kamolvisit W, et al. Exome sequencing as first-tier genetic testing in infantile-onset pharmacoresistant epilepsy: diagnostic yield and treatment impact. *Eur J Hum Genet*. 2022. doi: 10.1038/s41431-022-01202-x

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Spatial and cellular distribution of clonal hematopoiesis

Clonal hematopoiesis refers to the presence of somatic mutations in the blood cells of people who do not have cytopenias or diagnostic features of a hematopoietic malignancy. It has been associated with an increased risk of developing hematologic malignancies and cardiovascular disease. The authors conducted a study to better

characterize the genetic landscape of clonal hematopoiesis (CH) and the potential spatial and lineage-specific differences in the distribution of CH driver variants. They used targeted sequencing to analyze the mutation spectrum of CH in otherwise healthy people undergoing hip arthroplasty for osteoarthritis (n = 261). The authors also analyzed age-matched samples of myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia (sAML) for comparison purposes. They found that the allele frequencies of CH driver mutations in bone marrow samples were, overall, similar to those found in peripheral blood samples. However, in a subset of 21 people for whom paired femoral head bone marrow and peripheral blood samples were obtained simultaneously, the allele frequencies of 31 of 43 identified variants were slightly higher in the bone marrow compartment than in the corresponding peripheral blood sample. Yet in all CH cases with paired bone marrow and peripheral blood samples, next-generation sequencing (NGS) identified one or more variants in the peripheral blood specimen, indicating that when screening for CH, analyses of peripheral blood are sufficiently sensitive. The authors also analyzed 11 people who underwent bilateral simultaneous hip replacement to study the spatial heterogeneity of CH in the bone marrow compartment. They identified CH in eight of the 11 people. Comparison of the left and right femoral heads showed concordance in the detected variants in six of them. However, in two people, NGS identified an *ASXL1* variant on one side but not the contralateral side, suggesting intra-patient spatial heterogeneity. Flow-sorted populations were used to assess differential lineage distribution of *ASXL1* variants in CH in five patients with *ASXL1* variants. Overall, *ASXL1* allelic burden was significantly higher in bone marrow stem cells and myeloid progenitor cells. Most *ASXL1* variants demonstrated a variant allele frequency (VAF) in T-cell and B-cell populations of less than one percent, except the c.1934dupG variant, which showed a VAF of more than one percent in T-cell and B-cell populations. Over a median of 12 months of follow-up, longitudinal assessment of peripheral blood in CH patients showed a slight variation in VAF over time—but no patient showed strong clonal expansion. Age-matched cases of MDS and sAML showed a higher median number of mutations and a higher median VAF when compared to CH cases. Mutations in splicing factors, signaling pathways, and transcription factors were more commonly associated with MDS or sAML than with CH. While *DNMT3A* mutations were seen in CH, MDS, and sAML, CH cases showed fewer *DNMT3A* codon R882 missense mutations but a greater frequency of insertions/deletions, nonsense variants, or non-R882 missense *DNMT3A* variants. This study substantiates many of the findings about CH characteristics found in previous studies and provides further insight into the genetics and evolution of CH. In particular, the study findings highlight the potential heterogeneity of CH clone distribution and support the need to further investigate the spatial heterogeneity of CH and its clonal evolution into myeloid neoplasia.

Hartmann L, Hecker JS, Rothenberg-Thurley M, et al. Compartment-specific mutational landscape of clonal hematopoiesis. *Leukemia*. 2022. doi:10.1038/s41375-022-01700-3

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