Molecular pathology selected abstracts

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Genome sequencing as an alternative to cytogenetic analysis in myeloid cancers

June 2021—In myeloid malignancies, identification of genetic abnormalities informs diagnostic classification, aids risk stratification, and often predicts response to clinical therapy. Multiple methodologies generally are necessary to detect these abnormalities given the diversity of genetic occurrences, which can range from single-nucleotide variants to chromosomal translocations. Conventional metaphase cytogenetic analysis—that is, karyotyping—remains the standard of care for detecting chromosome-level abnormalities. In some instances, it is supplemented with FISH. Single-gene polymerase chain-reaction–based assays or next-generation sequencing studies, or both, are used to identify smaller genetic abnormalities, single-nucleotide variants, and smaller insertions/deletions. Whole genome sequencing (WGS) has not commonly been used with diagnostic acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) samples, in part due to the high cost of sequencing, complexity of interpretation, and long turnaround times. The authors conducted a study in which they developed a more streamlined approach to WGS for genomic profiling of patients with AML or MDS and applied that approach to diagnostic clinical samples to assess its feasibility, accuracy, and utility in the clinical setting. They employed a streamlined WGS approach to obtain genomic profiles for 263 patients with myeloid cancers, including 235 who had undergone successful cytogenetic analysis. Scalable sample-preparation methods that can be performed with commercially available reagents were used. Automated tumor-only variant analysis identified mutations in 40 genes, genomewide copy-number alterations greater than 5 Mbp, and recurrent structural alterations in myeloid malignancies. The authors compared WGS performance against conventional karyotyping and sequencing and used FISH, PCR, chromosomal microarray, and/or RNA-sequencing data to confirm WGS findings that were not detected by cytogenetic analysis. They risk-stratified patients according to European Leukemia Network (ELN) or International Prognostic Scoring System-Revised (IPSS-R) categories. In addition to detecting all 40 translocations and 91 copy-number alterations that had been identified by cytogenetic analysis, WGS identified new clinically relevant genomic events, including chromosomal translocation involving *CBFB-MYH11* and rearrangements involving *KMT2A*, in 40 (17 percent) of 235 patients with a known or suspected hematologic cancer. When compared to high-coverage (greater than 500×) targeted clinical sequencing, WGS demonstrated sensitivity of 84.6 percent for single-nucleotide variants and 91.5 percent for insertion-deletion mutations. False-negative findings were due to variants being present in a subclone or at low-coverage positions. In a prospective analysis of 117 consecutive patients, WGS provided additional genetic information in 29 (24.8 percent) patients and changed risk stratification in 19 (16.2 percent) patients, with a median turnaround time of 5.1 days. Risk-group assignments based on conventional testing were in agreement with risk-group assignments based on WGS for 63 of 71 (89 percent) patients. Five patients had new adverse-risk findings identified by WGS. In patients with previously inconclusive results by cytogenetic analysis, WGS detected risk-defining cytogenetic abnormalities, including a complex karyotype and rearrangements in *KMT2A* and *RUNX1-RUNX1T1,* in four patients. The remaining patients had a normal karyotype or one or two abnormalities identified by WGS. AML risk groups, as defined by WGS, correlated with clinical outcomes. Additional studies are necessary to confirm the authors' findings regarding the clinical validity of WGS as an alternative to conventional cytogenetic analysis in myeloid malignancies. However, this proof-of-concept study supports the potential clinical utility of WGS in simplifying testing algorithms, particularly as cost, time, and complexity of sequencing becomes less prohibitive. Such testing may also help detect clinically relevant abnormalities, particularly in cases for which conventional cytogenetics is not possible or is inconclusive.

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Use of genome sequencing analysis to identify new loci associated with Lewy body dementia

Lewy body dementia is a neurodegenerative disease characterized by a clinically heterogeneous spectrum of progressive cognitive decline, parkinsonism, and visual hallucinations. Autopsies on patients with Lewy body dementia (LBD) show widespread Lewy bodies and Lewy neurites (a hallmark feature of Parkinson disease), as well as Alzheimer disease copathology. This led to the hypothesis that LBD lies on a spectrum between Parkinson disease and Alzheimer disease. However, the precise genetic pathophysiology of LBD is poorly understood. To further characterize genetic contributions to this disorder, the authors performed whole genome sequencing (WGS) in a cohort of 2,981 patients diagnosed with LBD and 4,391 neurologically healthy people. They analyzed the data using a genomewide association study (GWAS) and gene-aggregation tests, colocalization analysis, and modeling of potential contributions of Alzheimer disease and Parkinson disease risk variants to LBD. The authors identified five loci that surpassed the genomewide significance threshold, three of which were located at known LBD risk loci within the genes *GBA, APOE,* and *SNCA*. They also identified a new LBD locus on chromosome 2q14.3, located downstream of the *BIN1* gene (a known risk locus for Alzheimer disease), and a second LBD locus within the *TMEM175* gene on chromosome 4p16.3 (a known Parkinson disease risk locus). The authors replicated each of the observed risk loci in an independent sample of 970 LBD patients with European ancestry and 8,928 control subjects. Gene-level aggregation testing revealed that *GBA*, a known risk gene for Parkinson disease, is a significant locus in LBD development. The authors found strong cis-eQTL (expression quantitative trait loci) colocalization signals at the *TMEM175* and *SNCA-AS1* loci, suggesting that changes in the expression of these genes may impact LBD disease risk. They used WGS data to explore the relationship between Alzheimer disease, Parkinson disease, and LBD. Genetic risk scores derived from large-scale GWAS analyses of Alzheimer disease and Parkinson disease were applied to individual-level genetic data from the LBD case-control cohort. Those diagnosed with LBD had a higher genetic risk for developing Alzheimer disease (odds ratio [OR], 1.66; 95 percent confidence interval [CI], 1.58–1.74; $P < 2 \times 10^{-16}$) and Parkinson disease (OR, 1.20; 95 percent CI, 1.14–1.26; $P = 4.34 \times 10^{-12}$). These risk scores remained significant after adjusting for genes that contribute substantially to Alzheimer disease (*APOE*) and Parkinson disease heritable risk (*GBA, SNCA,* and *LRRK2*). This study identifies relevant genetic loci in LBD and highlights the considerable overlap between genes associated with LBD and genes implicated in Parkinson disease and Alzheimer disease. This supports the hypothesized relationship between LBD and these related disorders. However, the authors note that this study was restricted by LBD cohorts that were heavily weighted towards people of European ancestry, a lack of in-depth phenotype information for most study participants, and limited study power to detect common genetic variants of small effect size. Larger studies involving more diverse cohorts are necessary to confirm the authors' findings.

Chia R, Sabir MS, Bandres-Ciga S, et al. Genome sequencing analysis identifies new loci associated with Lewy body dementia and provides insights into its genetic architecture. *Nat Genet*. 2021;53(3):294–303.

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