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Optical Genome Mapping and Long Read Sequencing: Mirror of the Genome

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Assessment of Optical Genome Mapping for Analysis of Structural Variants in Constitutional Postnatal Cases (Iqbal et al., 2023)

This study compared current standard of care (SOC) methods used in clinical cytogenetics (chromosomal microarray, karyotyping, fluorescent in situ hybridization, Southern blot analysis, and PCR) with optical genome mapping (OGM). The authors evaluated a total of 409 samples (50 negative controls and 359 individuals with suspected genetic disorders who were referred for cytogenetic testing. Structural variants including copy number variants (CNVs), aneuploidies, regions of homozygosity, contractions in facioscapulohumeral dystrophy 1, and repeat expansion in *FMR1* were analyzed. The American College of Medical Genetics and Genomics (ACMG) guidelines were used for the classification of variants. Intrasite and intersite reproducibility, concordance of technical and clinical classification, and ability to provide additional clinically relevant information were used as measures for comparing OGM with SOC. Majority of the samples (98%) yielded successful data for interpretation and analysis. Technical concordance with OGM was 99.5% and replicative analysis showed 100% concordance. Blinded analysis and variant classification agreement was 97.6% between SOC and OGM. Thus, the authors concluded that OGM is an alternative to SOC for rapid diagnosis of postnatal constitutional disorders due to its analytic validity and clinical utility.

16p13.11p11.2 triplication syndrome: a new recognizable genomic disorder characterized by optical genome mapping and whole genome sequencing (Nicolle et al., 2022)

The short arm of chromosome 16 contains several highly identical segmental duplications (SDs). These account for over 5% of the human genome. Non-allelic homologous recombination (NAHR) leads to recurrent chromosomal rearrangements. SDS are susceptibility factors for these rearrangements. Several genomic disorders involving 16p have been identified by chromosomal microarray (CMA). However, there are limitations to the resolution of CMA and short reads of whole genome sequencing (WGS). These are major hurdles in the characterization of more complex chromosomal rearrangements. In this study, the authors have reported two unrelated patients with de novo 16p13.11p11.2 triplication with a 16p11.2 duplication detected by CMA. The two patients had similar clinical features (hypotonia, severe developmental delay, hyperactive behaviour, facial dysmorphism, and conductive hearing loss). The rearrangement breakpoints could not be mapped precisely with short-read WGS. Thus, optical genome mapping (OGM) was used to determine the genomic positions of breakpoints and relative orientation of triplicated and duplicated segments. Thus, the authors identified a mechanism involving recombination between allelic SDs and an NAHR event and reported a new genomic disorder. They concluded that OGM can be used to detect mechanisms of complex chromosomal rearrangements involving SDs.

High diagnostic potential of short and long read genome sequencing with transcriptome analysis in exome–negative developmental disorders

(Lecoquierre et al., 2023)

Nowadays, exome sequencing (ES) is the method of choice for the diagnosis of rare diseases. The authors conducted a pilot study of five individuals with neurodevelopmental disorders (NDD). They performed trio-based short-read genome sequencing (srGS), long-read genome sequencing (lrGS), and case only transcriptome sequencing (TS) and identified three new genetic variants in three individuals. A case of Perching syndrome caused by a homozygous deep intronic variant in the *KLHL7* gene resulting in a neo-exon inclusion was identified by srGS. A case of Sotos syndrome which had a balanced inversion in *NSD1* was identified by lrGS. A de novo mosaic intronic 22-bp deletion in *KMT2D* causing Kabuki syndrome was also identified by srGS. TS of these 3 cases showed monoallelic expression, decreased gene expression, and splicing defects respectively, thus validating the effect of these variants. The study highlights the utility and complexities of these technologies.

Diagnosis of Prader–Willi syndrome and Angelman syndrome by targeted nanopore long–read sequencing

(Yamada et al., 2022)

Detection of abnormal methylation in the promoter of *SNRPN* is the basis of molecular diagnosis of PWS and AS. Nanopore sequencing is a unique, scalable technology that enables direct, real-time analysis of long DNA or RNA fragments. It works by monitoring changes to an electrical current as nucleic acids are passed through a protein nanopore. CpG methylation is detected through differences in electrical current intensities produced from nanopore reads of unmethylated and methylated bases. The authors successfully diagnosed four Prader-Willi syndrome patients and three Angelman syndrome patients by targeting differentially methylated regions. Concurrent copy number analysis, homozygosity analysis, and structural variant analysis enabled precise delineation of the underlying pathogenic mechanisms, including gross deletion, uniparental heterodisomy, uniparental isodisomy, or imprinting defect.

References

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