

Enhancing Short-Read Genome Sequencing Using Optical Genome Mapping and Long-Read Sequencing

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Introduction & Methods

- Genome sequencing (GS) has become a key diagnostic tool in the evaluation of individuals with rare diseases.
- GS has been shown to establish molecular diagnoses at higher rates than targeted testing while reducing the need for sequential testing approaches.^{1,2}
- Because GS can increase opportunities for more informed clinical management, GS is now recommended as a first-tier test for common clinical indications, such as intellectual disability and epilepsy, by professional and medical societies.³⁻⁵
- While GS is more comprehensive than targeted approaches, over 50% of individuals with a suspected genetic condition still do not receive a definitive genetic diagnosis through GS.

- Emerging technologies are increasingly used by clinical diagnostic laboratories to supplement short-read GS.
- These include optical genome mapping (OGM), which allows for detection of complex structural variants that are often missed by sequencing-based approaches,⁶ and long-read sequencing, which more accurately captures short tandem repeat (STR) expansions⁷—limitations of current short-read GS.
- In March 2026, our clinical laboratory integrated OGM and long-read sequencing for assessment of these variants into our GS workflow.
- Here, we demonstrate the utility of these two technologies in enhancing the diagnostic capability of GS at our clinical laboratory.

Results

Optical Genome Mapping

Figure 1. OGM enables structure-level resolution and characterization of complex structural variants which are difficult to detect on short-read GS.

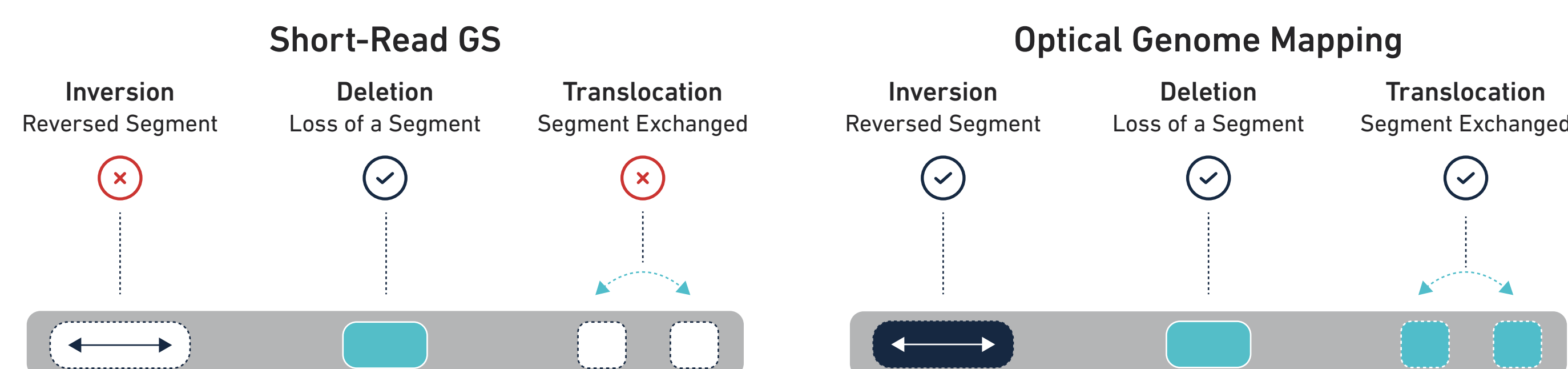
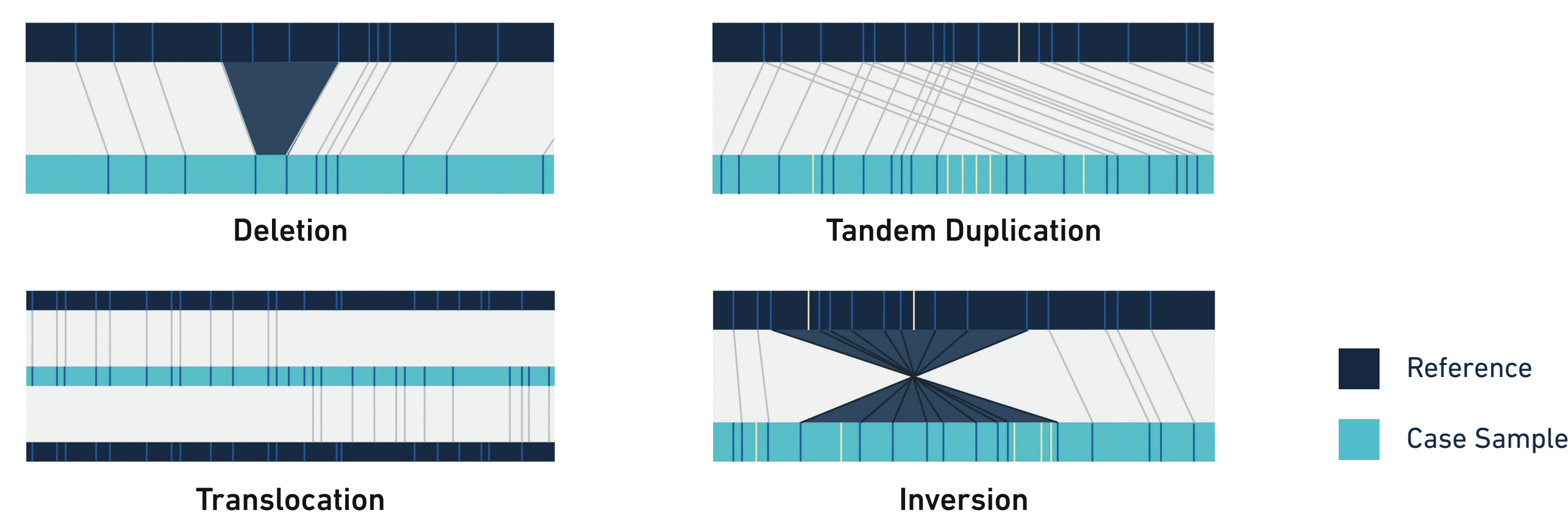
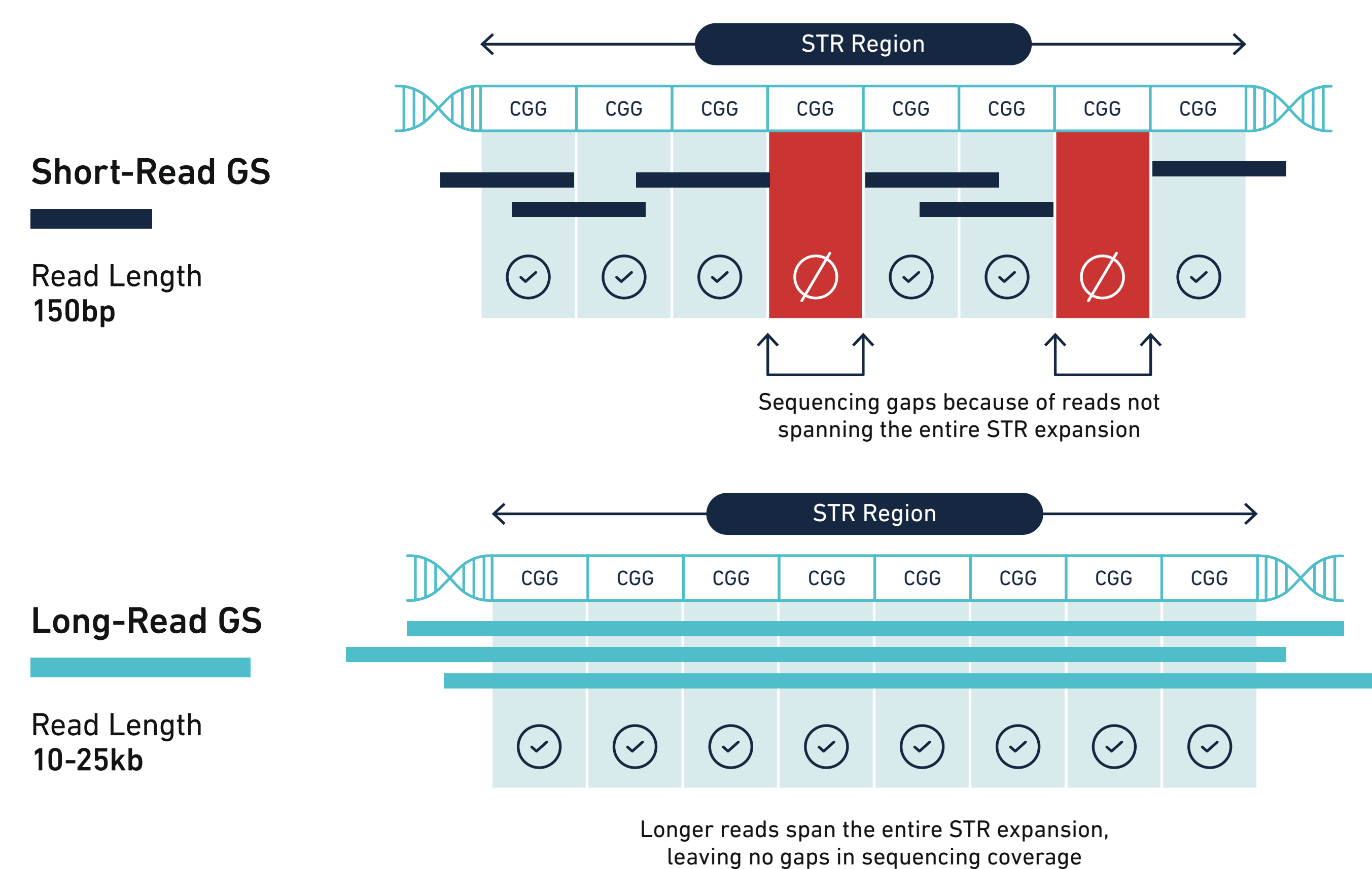


Figure 2. Visualization of different complex structural variants detectable by OGM analysis.



Long-Read Sequencing

Figure 7. STR expansion analysis enabled by long-read sequencing technology.



Case 1. OGM provides additional information following the identification of deletions on chromosome 9 and chromosome 14 through short-read GS.

Patient Presentation: Child with global developmental delay and hypotonia.

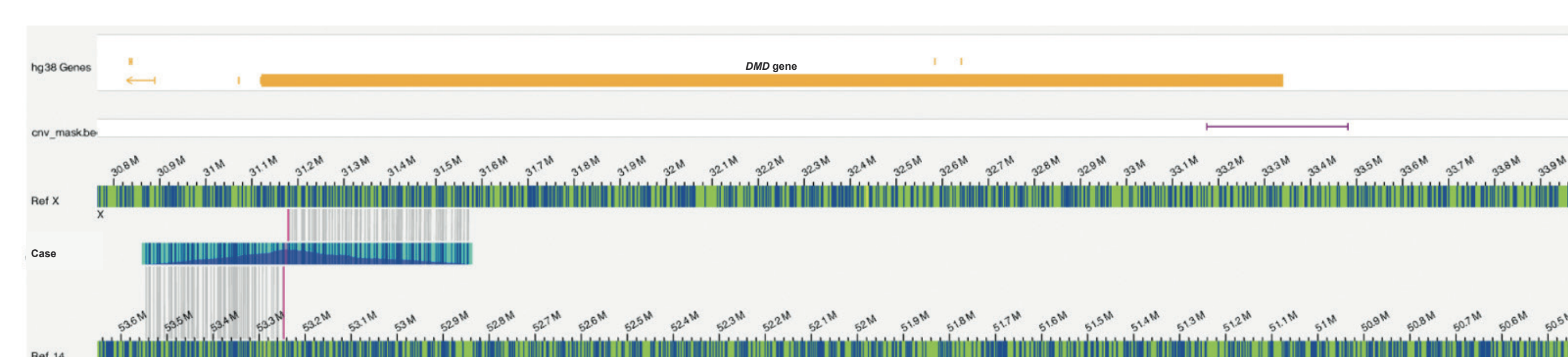


Figure 3. Distal breakpoint on chromosome 14 is translocated with the X chromosome directly into the *DMD* gene.

Result: OGM analysis demonstrated chromothrypsis/chromoplexy involving chromosomes 14, X, and 9, as well as a translocation breakpoint within the *DMD* gene and four inversions on chromosome 14. Highly skewed X-inactivation would be expected.

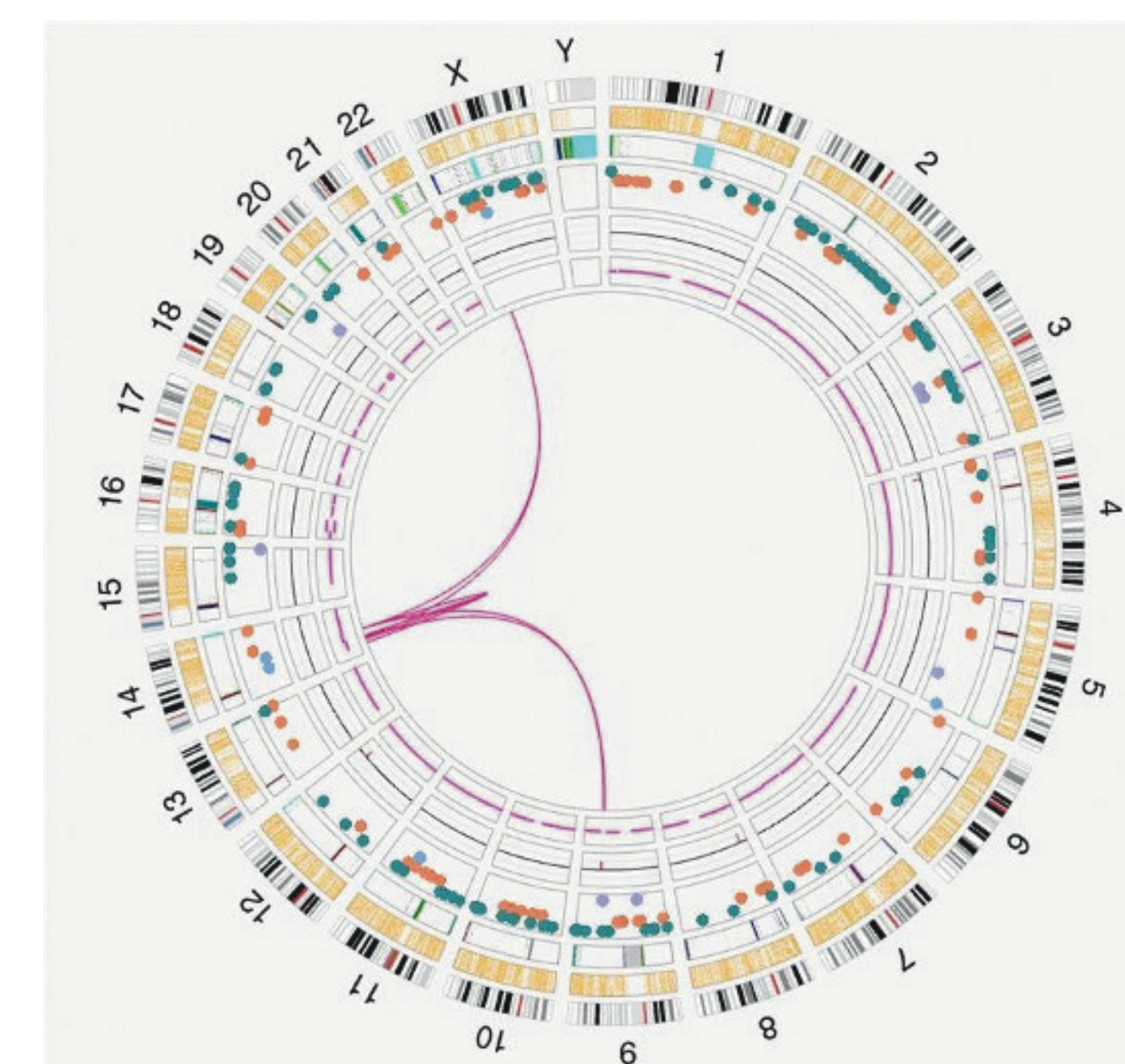


Figure 4. Circos plot of OGM analysis results.

Case 2. OGM provides additional information following the detection of multiple copy number losses on chromosome 1 through short-read GS.

Patient Presentation: Child with global developmental delay, hypotonia, and dysmorphic features.

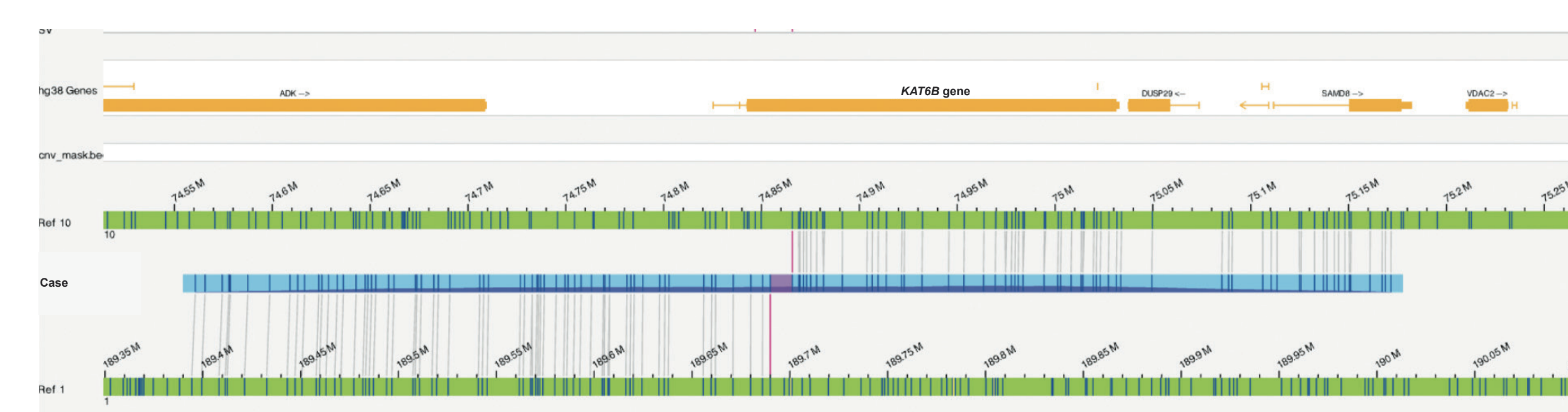


Figure 5. Breakpoint on chromosome 10, which translocates with chromosomes 1 and 9, is located within the *KAT5B* gene.

Result: OGM analysis demonstrated chromothrypsis/chromoplexy involving chromosomes 1, 2, 3, 9 and 10. A diagnosis of autosomal dominant *KAT5B*-related multiple congenital anomalies syndrome is likely due to breakpoint location within the *KAT5B* gene.

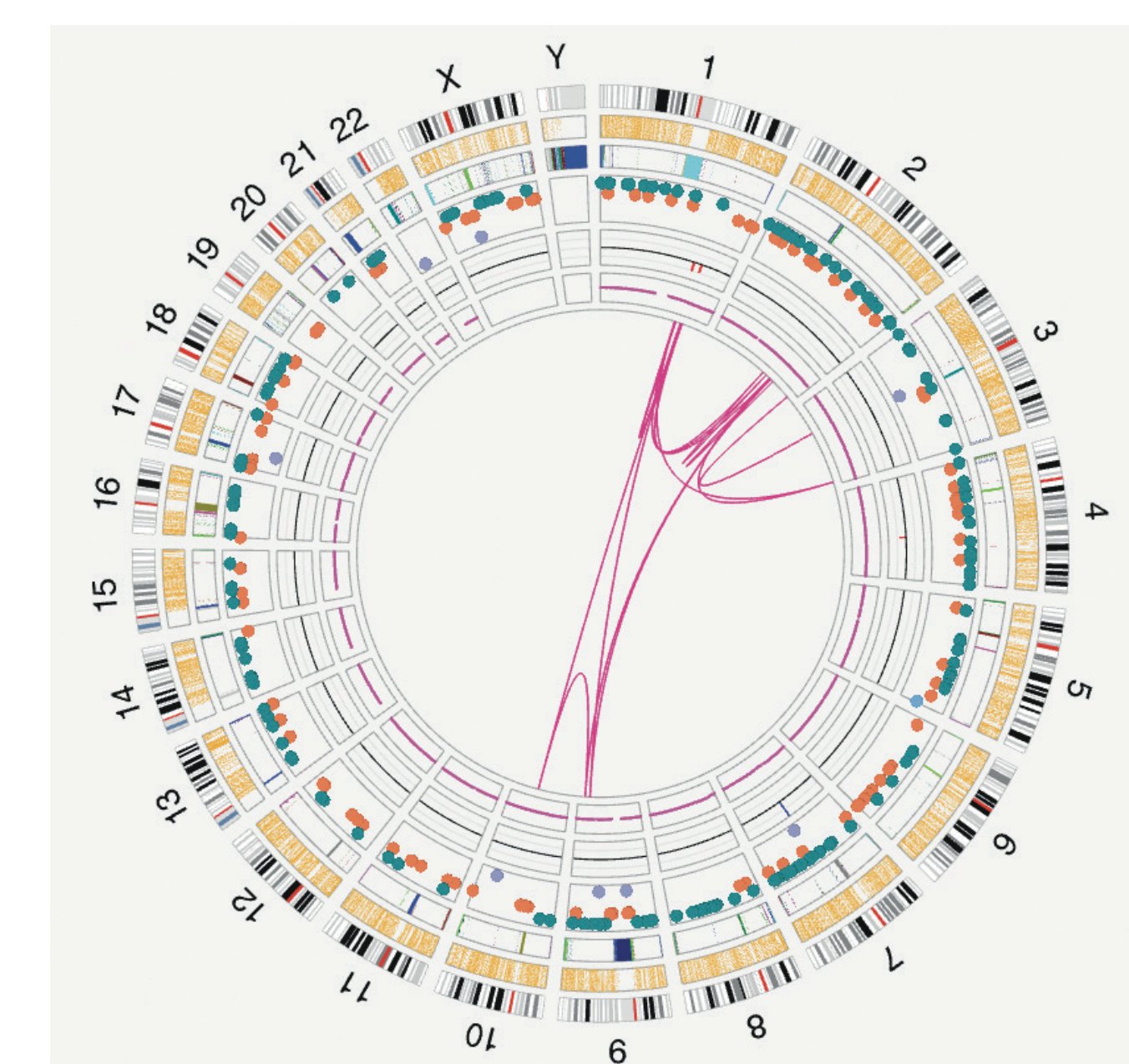


Figure 6. Circos plot of OGM analysis results.

Case 3. Long-read sequencing identified biallelic pathogenic repeat expansions in *RFC1*.

Patient Presentation: Adult with ataxia and neuropathy.

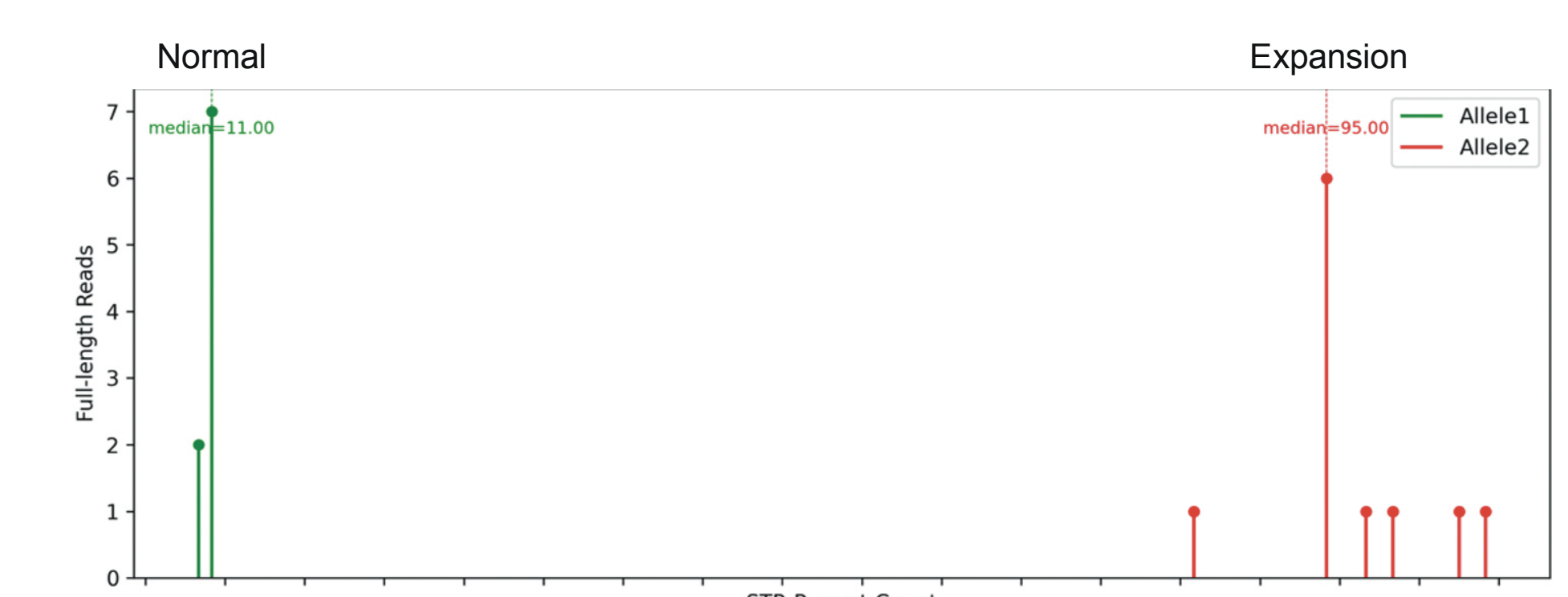


Result: Established diagnosis of autosomal recessive cerebellar ataxia, neuropathy, and vestibular areflexia (CANVAS) syndrome.

Case 4. Long-read sequencing clarified number of repeats in *GIPC1*.

Patient Presentation: Adult with ataxia, tremor, and polyneuropathy.

Figure 8. Histogram demonstrating one normal allele and one pathogenic repeat expansion of approximately 95 CGG repeats in *GIPC1*.



Result: Established diagnosis of autosomal dominant oculopharyngodistal myopathy 2.

Conclusions

- OGM has enabled the identification of structural variant breakpoint locations within critical genes, such as *DMD*, that are consistent with the clinical phenotype in several cases.
- In comparison to other orthogonal confirmation methods, long-read sequencing has been utilized to confirm and more accurately size STR expansions in genes such as *GIPC1* and *RFC1*.
- As these emerging technologies continue to be utilized to supplement short-read GS, large-scale studies will be necessary to further demonstrate their utility in the genetic evaluation of rare diseases and support access to these technologies.

References:

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