Optical Genome Mapping and Long Read Sequencing Identifies a Novel 27kb Dystrophin Gene Inversion in a Patient with Duchenne's Muscular Dystrophy

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INTRODUCTION

This study focuses on a male patient who presented to the clinic at five years of age with a compelling clinical picture for Duchenne's Muscular Dystrophy (DMD). His history included delayed motor milestones, frequent falls, and difficulty with stairs, and an examination revealed a positive Gower's sign. The clinical suspicion was strongly supported by a profoundly elevated Creatine Kinase (CK) level of 13,041 U/L and a muscle biopsy that confirmed severely reduced dystrophin labeling. Despite this evidence, the **patient faced a prolonged diagnostic journey as standard genetic testing, including a neuromuscular panel at age six and comprehensive *DMD* gene sequencing at age eleven, was repeatedly negative**, leaving his condition without a molecular confirmation. To further investigate this case, we performed both Optical Genome Mapping and Long Read Whole Genome Sequencing.

METHODS - LRS

To comprehensively analyze the *DMD* gene, we utilized two independent analysis methods: Long Read Sequencing was performed via the PromethION sequencing platform on R10.4.1 flow-cells from Oxford Nanopore Technologies to a targeted **30x whole-genome coverage, yielding average read lengths of 23kb**. Basecalling used Dorado (v.7.4.14). Structural variants were called via Sniffles (v2.4) and viewed in IGV (v.2.19.3). Inspection of the *DMD* gene in IGV revealed an inversion visualized by distinct breakpoints over a called (red bar) region across exons 68-73

RESULTS

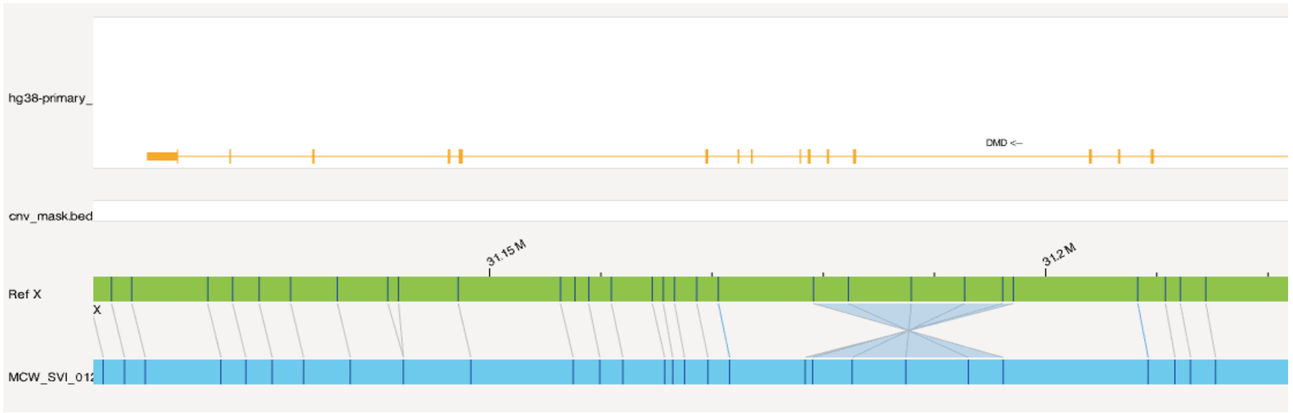

Figure 1: Optical Genome Mapping (Bionano) identifies a 27kb *DMD* inversion. Patient's *de novo* assembled genome map (blue track) is compared to the hg38 reference map (green track). The "bowtie" alignment pattern is the definitive signature of an inversion. This inversion includes exons 68-73.

Figure 2: Long Read Sequencing (ONT) confirms the 27kb inversion in the *DMD* gene. Reads with an average length of 23.19kb aligned to hg38 confidently display an inversion with breakpoints flanking exons 68-73. The red bar in this figure shows the range of the inversion with the edges being breakpoints. An inspection of the nucleotides at this location would reveal an inverse sequence compared to the reference.

METHODS - OGM

Orthogonally, Optical Genome Mapping was performed via the Saphyr System from Bionano, Inc. Variants were analyzed in the Bionano Access software (v1.8).
OGM was performed **achieving 252.4x average coverage with 335kb average fragment sizes**. A *de novo* assembly was generated using Bionano Access/Solve and compared against the hg38 reference to identify structural variants.

DISCUSSION

This case demonstrates that complex structural variants, such as small inversions, can be missed by standard short-read sequencing, leading to a prolonged diagnostic journey for patients with classic DMD. By leveraging Long Read Sequencing and Optical Genome Mapping, we successfully identified a 27kb pathogenic inversion in the *Dystrophin* gene, providing a definitive diagnosis. This novel 27kb inversion is a **previously unreported structural variant** in the *Dystrophin* gene. These results underscore the clinical utility of advanced genomic technologies in resolving complex cases. **We recommend that OGM and LRS should be considered for patients with strong clinical evidence of DMD when initial genetic testing is uninformative**, potentially preceding the need for more invasive procedures like a muscle biopsy.

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