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XIX Convegno Triennale sugli Aspetti Clinici e Sociali dell'Emofilia ABSTRACT N. CO-03

1.02 Genetics of congenital bleeding disorders (including prenatal diagnosis)

BEYOND CONVENTIONAL TESTING: NANOPORE LONG-READ SEQUENCING FOR RECURRENT F8 INVERSIONS IN HEMOPHILIA A

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Background and Aims. Haemophilia A is an X-linked bleeding disorder caused by pathogenic variants in F8. In severe disease, recurrent inversions involving intron 22 and intron 1 represent major molecular defects, but their detection and characterization remain challenging because of homologous repeated sequences and the structural complexity of the F8 locus. Conventional assays may have limited reproducibility and do not directly resolve the genomic architecture of the region. This pilot study aimed to validate an Oxford Nanopore Technologies long-read sequencing workflow for the direct characterization of recurrent F8 inversions. The project was developed with the support of the "Idee Giovani per la Ricerca Ed. 2025" award, with the goal of exploring long-read sequencing as a flexible approach for molecular diagnosis in Haemophilia A.

Materials and Methods. Three male patients with clinical Haemophilia A and recurrent F8 inversions were analyzed, including two cases carrying the intron 22 inversion and one case carrying the intron 1 inversion. Genomic DNA from peripheral blood was quality assessed and used for long-read library preparation. Sequencing was initially performed on MinION and subsequently optimized on PromethION using adaptive sampling. A dedicated Oxford Nanopore long-read analysis pipeline was developed and optimized for this study. Following Dorado basecalling, reads were aligned to the GRCh38 reference genome using minimap2. Structural variants were detected with Sniffles2 and SNVs/indels were called with Clair3. Variant annotation was performed using AnnotSV and ANNOVAR, while structural rearrangements were validated through manual breakpoint inspection in IGV.

Results. The workflow successfully resolved the molecular defect in all three patients. In the two intron 22 inversion cases, long-read sequencing confirmed the rearrangement, showed concordance with conventional molecular methods and enabled discrimination of the molecular inversion subtype. In the intron 1 inversion case supported direct interpretation of the affected genomic region. Overall, long-read sequencing allowed direct inspection of the F8 locus, supporting reconstruction of the rearranged architecture and orientation of the involved regions providing a comprehensive molecular assessment of the locus.

Conclusions. This pilot study demonstrates the feasibility of Nanopore long-read sequencing for the molecular diagnosis of Haemophilia A. The workflow enabled direct detection and characterization of recurrent F8 inversions, including intron 22 and intron 1 inversions, highlighting its added value over conventional approaches. This strategy provides a starting point for extending long-read sequencing to other forms of haemophilia and genetically complex inherited bleeding disorders.

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