IDENTIFIKASI VARIAN GENETIK DENGAN METODE *NANOPORE LONG-READ SEQUENCING* PADA SUBJEK DENGAN HEMOFILIA A

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INTISARI

Hemofilia A merupakan gangguan pembekuan darah yang disebabkan oleh mutasi gen *F8* yang mengakibatkan defisiensi faktor koagulasi VIII (FVIII). Gen *F8* yang terletak pada kromosom X memiliki panjang sekitar 186 kb dan mengandung 26 ekson. Struktur kompleks gen *F8* membuatnya rentan terhadap berbagai mutasi yang memengaruhi produksi FVIII. Oleh karena kompleksitas strukturnya, diperlukan metode yang tepat untuk mengidentifikasi mutasinya. *Long-read sequencing,* khususnya dengan *adaptive sampling* yang dikembangkan oleh *Oxford Nanopore Technologies* (ONT), memungkinkan identifikasi mutasi yang efisien pada gen berukuran besar seperti *F8*, sehingga meningkatkan akurasi diagnostik. Subjek dalam penelitian ini adalah anak laki-laki berusia 8 tahun dengan diagnosis Hemofilia A ringan (FVIII:C 34%). Identifikasi mutasi dilakukan menggunakan *Nanopore sequencing* dengan metode *adaptive sampling* dan *workflow wf-human-variation* pada platform EPI2ME. Hasil analisis menunjukkan kualitas data yang baik (total pembacaan: 214.408, *read* N50: 10.553 bp, *mean coverage*: 14,412x, *read quality*: 19,7, *mapping accuracy*: 98,4%, *coverage*: 93,5%). Pada ekson 10 gen *F8* ditemukan varian *missense* dengan signifikansi patogenik yaitu NM_000132.4:c.1492G>A (p.Gly498Arg), yang menyebabkan perubahan asam amino dari glisin menjadi arginin pada posisi 498. Selain itu, teridentifikasi empat varian gen (*THOC2*, *XIAP*, *INTS6L*, dan *MTM1*) dengan klasifikasi varian *uncertain significance*.

KATA KUNCI: Hemofilia A, varian patogenik, p.Gly498Arg.

IDENTIFICATION OF GENETIC VARIANTS BY NANOPORE LONG-READ SEQUENCING METHOD IN SUBJECT WITH HEMOPHILIA A

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ABSTRACT

Hemophilia A is a blood clotting disorder caused by a mutation of the *F8* gene that results in coagulation factor VIII (FVIII) deficiency. The *F8* gene, located on the X chromosome, is approximately 186 kb long and contains 26 exons. The complex structure of the *F8* gene makes it susceptible to various mutations that affect FVIII production. Due to the complexity of its structure, an appropriate method is required to identify its mutations. Long-read sequencing, particularly with adaptive sampling developed by Oxford Nanopore Technologies (ONT), enables efficient identification of mutations in large genes such as *F8*, thereby improving diagnostic accuracy. This study's subject was an 8-year-old boy diagnosed with mild Hemophilia A (FVIII:C 34%). Mutation identification was performed using Nanopore sequencing with adaptive sampling method and wf-human-variation workflow on EPI2ME platform. The analysis results showed good data quality (total reads: 214,408, read N50: 10,553 bp, mean coverage: 14.412x, read quality: 19.7, mapping accuracy: 98.4%, coverage: 93.5%). In exon 10 of the *F8* gene, a missense variant with pathogenic significance was found, namely NM_000132.4:c.1492G>A (p.Gly498Arg), which causes an amino acid change from glycine to arginine at position 498. In addition, four gene variants (*THOC2*, *XIAP*, *INTS6L*, and *MTM1*) were identified with variant classification of uncertain significance.

KEY WORDS: Hemophilia A, pathogenic variant, p.Gly498Arg.