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Prof Johannes Attems

Editor-in-Chief

Acta Neuropathologica

We kindly ask you to consider our manuscript titled **«Comprehensive evaluation of *MGMT* promotor methylation by nanopore sequencing»** for publication as an original article in Acta Neuropathologica. We confirm that this work is original and is not under consideration for publication in other journals.

Our manuscript describes how nanopore long-read sequencing can be used for comprehensive methylation analysis of the *MGMT* promoter region using native tumor DNA. We compare results obtained via nanopore sequencing to those generated by pyrosequencing and methylation bead array in a total of 148 CNS tumors, including 91 glioblastomas. We show that unsupervised hierarcical clustering of samples based on nanopore sequencing accurately separates methylated and un-methylated samples and this separation correlates with patient survival.

*MGMT* promoter methylation as a prognostic factor in glioblastoma patients is an active field of research. Several methods exist to measure MGMT promoter methylation, but nanopore sequencing is unique in detecting methylation on every CpG site in the region without the need for bisulfite treatment or amplification. It offers a complete and unbiased overview of *MGMT* promoter methylation that can be generated quickly and cost-effectivly. To the best of our knowledge, this is the first time all 98 CpG sites within the *MGMT* CpG island in multiple patient biopsies are analyzed by nanopore sequencing. We believe our findings will be of interest to the readers of Acta Neuropathologica.

All co-authors have read and approved of its submission to this journal.

Sincerely,

Dr. Skarphéðinn Halldórsson

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Norway