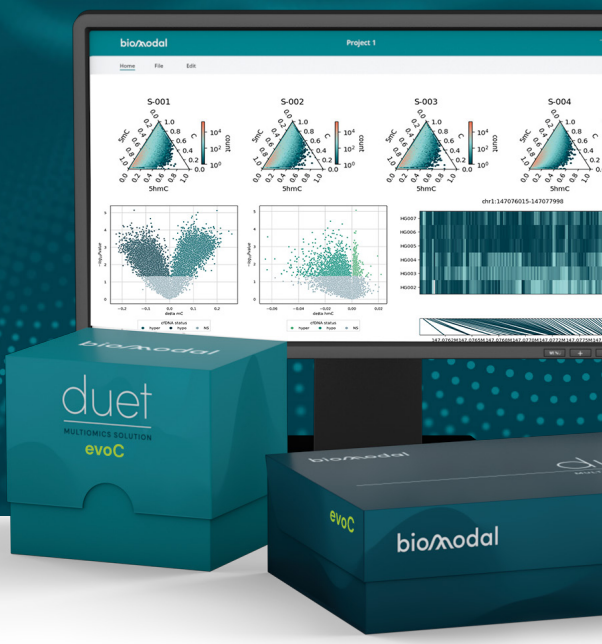
biomodal | Karolinska Institutet

CASE STUDY

Karolinska Institutet Researchers Use 6-Base Sequencing Technology to Decode Multiple Sclerosis



Overview

Early detection of multiple sclerosis (MS) is critical for treating to preserve brain tissue, yet most patients are diagnosed years after symptoms begin, and therapeutic options for progressive stage of disease are limited.

To address this gap, Maja Jagodic, Professor of Neuroinflammation at Karolinska Institutet, and her research group are combining genomic and epigenomic analyses to uncover how genetics, environment, and lifestyle interact in the onset and progression of MS. They aim to explain the molecular mechanism of MS and develop prognostic biomarkers for early diagnosis and prognosis.

Central to this effort is analyzing cell-free DNA (cfDNA) from cerebrospinal fluid (CSF) taken from MS patients, which provides a direct view of the neuronal and glial injury that occurs in MS but faces technical challenges.

"We very often deal with extremely low-yield cfDNA samples," explains Jagodic. "Before working with biomodal's 6-base sequencing technology, detecting 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) modifications required splitting our material into multiple batches. That was not an option, because we had so little to begin with."

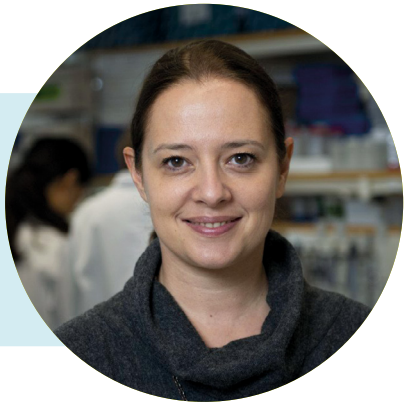
By adopting biomodal's 6-base sequencing solution, duet evoC, the researcher group at Jagodic's lab can now detect both modifications sensitively with as little as 50 picograms of input.

Results with duet evoC at Karolinska Institutet

- Genomic, 5mC, and 5hmC analysis in a single workflow
- Discriminate neuronal DNA signatures using 5mC and 5hmC data
- Compatible with ultra-low inputs, from 50 pg to 2 ng of cfDNA
- Excellent support for data analysis and protocol troubleshooting

"Learning more about the underlying biology or identifying potential biomarkers from epigenetic profiling of CSF will be quite groundbreaking in neurology."

Maja Jagodic, Professor of Neuroinflammation at Karolinska Institutet



Early results reveal neuron-specific 5hmC signatures enriched in pathways linked to neurogenesis and axon development, with potential as early biomarkers for MS. Looking ahead, the team aims to refine targeted sequencing approaches and ultimately translate these findings from CSF to blood for more patient-friendly biomarker detection.

These advances lay the foundation for earlier diagnosis, a better mechanistic understanding of disease progression, and ultimately, more effective treatment strategies for MS.

The Jagodic Lab: Epigenetics and its Role in MS Progression

MS is an autoimmune disease that occurs when the immune system attacks the myelin sheath, causing neuronal cell death and lesions throughout the brain. Most patients experience a relapsing-remitting course that gradually worsens, making early symptoms hard to recognize and disease progression unpredictable.

Because therapies are most effective when administered early, delayed diagnosis poses a significant challenge. “People usually have had the disease for several years, sometimes 5-10 years, before they get diagnosed,” explains Jagodic. “By the time we diagnose MS, we’ve lost valuable time to prevent brain damage.”

Jagodic’s Lab aims to change this through the study of the complex molecular underpinnings of the disease, using these insights to develop early diagnostic and prognostic biomarkers. “We know there are several genetic variants that increase the susceptibility to developing MS,” Jagodic continues, “but there are also environmental factors or lifestyle exposures that are involved as well.”

To address this interplay, the group uses epigenetic analysis.

“One reason we became interested in epigenetics is that it represents the convergence of both genetic background and environmental exposures,” says Jagodic. “These combined factors shape how cells use their genomes, ultimately affecting cell function and phenotype.”

Where earlier studies focused mainly on genome-wide associations, Jagodic’s team now integrates genetic and epigenetic information to identify new disease links. “Now, we can combine genomic and epigenetic information to help us discover the downstream effects of a genetic variant, along with the activity of different cell types due to their unique epigenetic signature,” remarks Jagodic.

By combining these approaches, the lab aims to develop new diagnostics that enhance MS detection, management, and treatment globally.

> “Nowadays, studying genetics without the epigenetic layer is an oversimplified approach to studying disease.”
>
> Maja Jagodic,
> Professor of Neuroinflammation at Karolinska Institutet

The Challenge: Distinguishing 5mC from 5hmC in Ultra-Low Yield cfDNA Samples

To perform genetic and epigenetic analyses, Jagodic’s group uses CSF collected from MS patients, because it is in direct contact with the brain and contains cfDNA fragments released during neuronal or glial cell death.

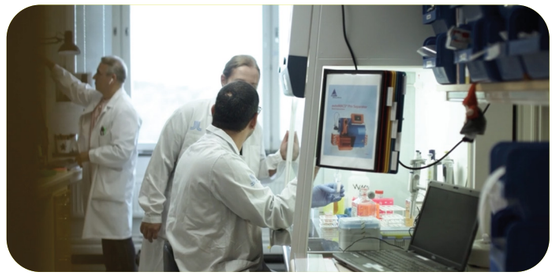
“We can analyze fresh material but also archival clinical samples, because the DNA modifications we are looking for, 5mC and 5hmC, are remarkably stable,” Jagodic comments. “This allows us to study larger patient cohorts and explore how genetic variants and environmental influences shape disease onset and progression.”

CSF is also considered “cleaner” compared to other cfDNA sources, such as plasma, as it has less contribution from other peripheral tissues, making it more specific for detecting brain-specific genetic and epigenetic signatures.

While CSF is biologically and clinically relevant, one significant limitation is that cfDNA concentrations in CSF are extremely low. Low yields make it incompatible with conventional epigenetics methods, such as bisulfite sequencing, which exposes DNA to harsh chemical treatments that can lead to DNA damage.

Other methods are more suitable, yet present different challenges.

“Enzymatic sequencing works, even with our low input of cfDNA,” says Jagodic. “But we only get bulk signal from methylation and hydroxymethylation. We know from analysis of post-mortem tissue from MS patients that the hydroxymethylation signal is significant; not being able to distinguish between 5mC and 5hmC means we are losing valuable information.”



Deconvoluting these different epigenetic modifications from one another requires splitting material into separate workflows, an approach that is impossible with such limited input. This barrier has prompted the group to search for other enabling technologies that extract the maximum amount of genetic and epigenetic information from their low-yield samples.

The Solution

While searching for a better solution for genetic and epigenetic analysis from their low-yield samples, the Jagodic Lab discovered biomodal’s 6-base sequencing technology.

“We got very excited when we learned about duet evoC at one of our conferences,” Jagodic remembers. “It was really a momentous moment to see the development of this methodology for simultaneous detection of all six bases.”

The product is uniquely suited for solving the Jagodic research group’s problem: With 5 ng of input DNA and a single workflow, the technology can distinguish traditional DNA bases (A, T, C, and G) and two modified cytosines, 5mC and 5hmC.

Method

The Jagodic Lab used duet evoC to analyze epigenomic signatures in cfDNA isolated from the CSF of MS patients.

“The whole premise of our workflow,” says Jagodic, “is that we can use the epigenetic mark that is specific for neurons or glial cells to identify the debris coming from these cells when they’re dying and the cfDNA is being shed into the CSF.”

The group also analyzed cfDNA from CSF samples taken from traumatic brain injury (TBI) patients, which act as a positive control, as they have more neural and glial cells that are damaged or dying, and cfDNA, compared to a healthy control.

Using the duet software and the support of technical experts at biomodal, Jagodic’s group was able to analyze the individual 5mC and 5hmC signatures from neuronal and glial DNA.

Results

To validate duet evoC, Jagodic's group isolated genomic DNA from immune cells in CSF, primary neurons, glial cells, and cerebellar tissue (provided by the biomodal team). These samples enabled genome-wide quantification of 5hmC, with most 5hmC modifications clustering around enhancers and gene bodies (for both coding and non-coding genes). This initial experiment also helped them identify neuron-specific 5mC and 5hmC signals, allowing them to deconvolute the 5hmC signal in CSF-derived cfDNA with greater sensitivity.

> "The preliminary results look very interesting and promising so far," Jagodic notes. "It's not just the technology itself, but also the wonderful collaboration with the biomodal team that made it possible to address many analytical questions and refine our model."

To identify neuron-specific, injury-associated differentially hydroxymethylated regions (DhMRs), the team utilized CSF samples from people who suffered TBI and compared them to healthy individuals uncovering 5hmC in genes enriched in axon development, neurogenesis, and neuronal differentiation pathways. Notably, neuron-specific 5hmC signatures were elevated in cfDNA from the CSF of MS patients. Even with as little as 50 pg of input, this signal was detectable, enabling multiple analyses from precious, yet limited CSF-derived cfDNA.

Future Directions

The next step for the Jagodic Lab is to combine 5mC and 5hmC signals to improve the detection of neuron-derived cfDNA from CSF samples. In addition, exploring the DhMRs throughout the progression of MS may shed light on early predictive MS biomarkers and the impact of genomics on epigenomics throughout the course of the disease.

The identification of a neuron-specific epigenetic signature also enables the group to take a more focused sequencing approach, using target capture technology to enrich existing sequencing libraries for only the loci that are most informative to increase sensitivity, allowing them to make the best use of their low-yield cfDNA samples.

Finally, the group aims to translate their findings from CSF to blood, which is considered more patient-friendly as it's collected through a blood draw. CSF collection, by contrast, requires an invasive lumbar puncture, which carries higher risks and greater discomfort.

"In conditions like MS, the blood-brain barrier is compromised so that cfDNA fragments could make their way into plasma," says Jagodic. "If we manage to get a very specific way of seeing 5mC and 5hmC in these fragments, it will be much better for clinical biomarker detection."

Jagodic believes in the impact of the work her lab is doing with biomodal: "Learning more about the underlying biology or identifying potential biomarkers from epigenetic profiling of CSF will be quite groundbreaking in neurology."
