Study reveals significant differences in RNA editing between postmortem and living human brain

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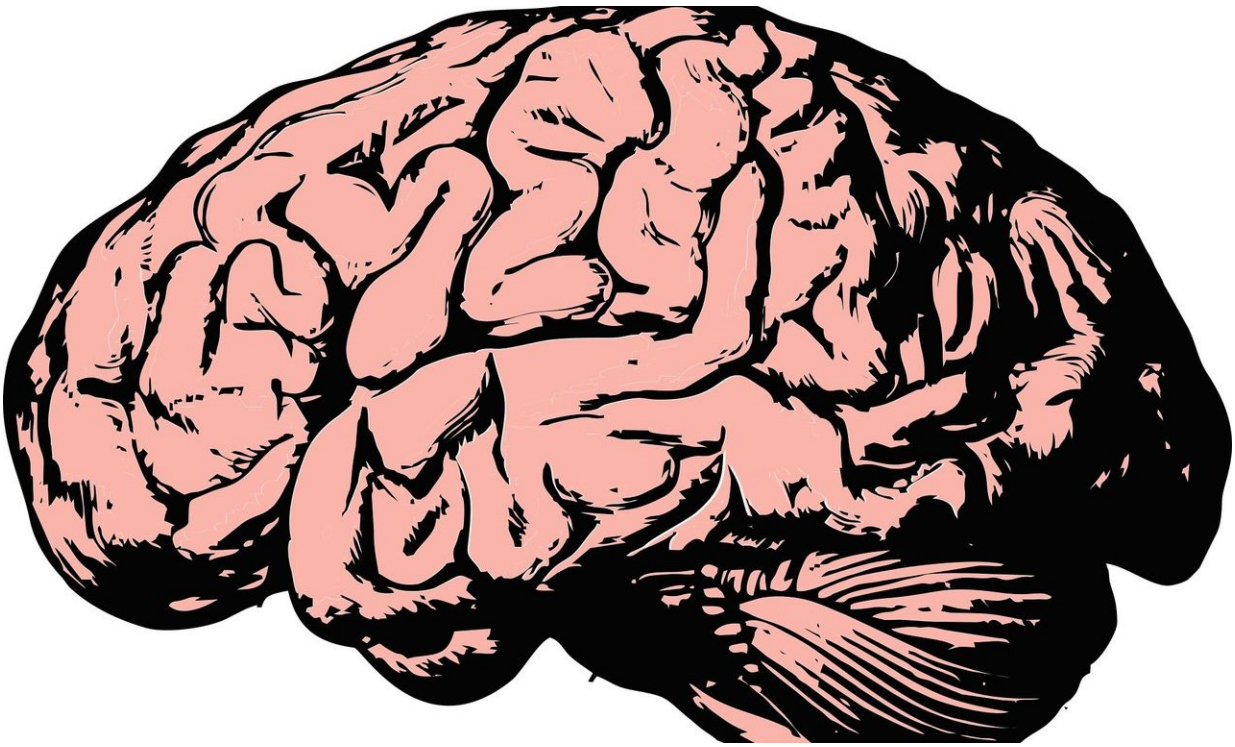

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Researchers from the Icahn School of Medicine at Mount Sinai have shed valuable light on the nuanced functions and intricate regulatory methods of RNA editing, a critical mechanism underlying brain

development and disease.

In a study published June 26 in *Nature Communications*, the team reported finding major differences between postmortem and living prefrontal cortex brain tissues as they relate to one of the most abundant RNA modifications in the brain, known as adenosine-to-inosine (A-to-I) editing.

This discovery will play a significant role in shaping the development of diagnostics and therapies for brain diseases.

While DNA holds the genetic blueprint for humans, RNA actually carries out its instructions to create functional proteins that play important roles in how the body functions, including the complex functions of the central nervous system. RNA's function and stability are controlled by many modifications, each holding a specific purpose.

These modifications, known as RNA editing, are a continuous process occurring in all our cells and tissues, facilitated by enzymes known as ADAR. This process can continue to occur in individual cells for some time after the death of the person whose tissues the cells were part of.

The conversion of adenosine nucleosides to inosine (A-to-I) is a common and well-studied RNA modification and is orchestrated by proteins in the ADAR family, primarily ADAR1 and ADAR2.

In the mammalian brain, thousands of highly regulated A-to-I editing sites have been discovered across anatomical regions and cell types, some by Mount Sinai researchers. These sites are known to be involved in neuronal maturation and brain development. Aberrant regulation of A-to-I editing has been linked to neurological disorders.

"Until now, the investigation of A-to-I editing and its biological

significance in the mammalian brain has been restricted to the analysis of postmortem tissues. By using fresh samples from living individuals, we were able to uncover significant differences in RNA editing activity that previous studies, relying only on postmortem samples, may have overlooked," said Michael Breen, Ph.D., co-senior author of the study and Assistant Professor of Psychiatry, and Genetics and Genomic Sciences, at Icahn Mount Sinai.

"We were particularly surprised to find that RNA editing levels were significantly higher in postmortem brain tissue compared to living tissue, which is likely due to postmortem changes such as inflammation and hypoxia that do not occur in living brains.

"Additionally, we discovered that RNA editing in living tissue tends to involve evolutionarily conserved and functionally important sites that are also dysregulated in human disease, emphasizing the need to study both living and postmortem samples for a comprehensive understanding of brain biology."

After death, the lack of oxygen quickly damages brain cells, causing an irreversible cascade of damage that can alter ADAR expression and A-to-I editing.

"We hypothesized that molecular responses to postmortem-induced hypoxic and immune responses can significantly alter the landscape of A-to-I editing. This can lead to misunderstandings about RNA editing in the brain if we only study postmortem tissues," said Miguel Rodríguez de los Santos, Ph.D., co-first author of the study and a postdoctoral fellow in the Department of Psychiatry at Mount Sinai.

"Studying living brain tissue provides us with a clearer picture of RNA editing biology in the human brain."

To investigate, the research team anchored their study around the Living Brain Project, in which dorsolateral prefrontal cortex (DLPFC) tissues from living people are obtained during neurosurgical procedures for deep brain stimulation, an elective treatment for neurological illness.

For comparison, a cohort of postmortem DLPFC tissues across three brain banks was assembled to match the living cohort for key demographic and clinical variables. The team investigated multiple genomic data types from the Living Brain Project, including bulk tissue RNA sampling, single-nuclei RNA sequencing, and whole-genome sequencing. The generation of this data is being described in multiple forthcoming Living Brain Project manuscripts.

The researchers identified more than 72,000 locations where A-to-I editing occurs more often or differently in postmortem than in living DLPFC brain tissue. They found higher levels of the enzymes ADAR and ADARB1, which are responsible for elevated editing patterns in postmortem brain tissues. Interestingly, they also found hundreds of sites with higher levels of A-to-I editing in living brain tissue.

These sites are mostly found in the connections between neurons (called synapses) and are typically conserved through evolution, suggesting they play important roles in brain activity.

Some well-known A-to-I editing sites were highly edited in living brains, indicating they may be involved in critical neuronal processes like synaptic plasticity, which is essential for learning and memory. However, many other A-to-I editing sites found in living brain tissues have unclear functions, and further research is needed to understand their impact on brain health.

"Utilizing fresh brain tissue from living human donors provided us the opportunity to investigate the brain without the confounds inherent to

postmortem tissue analysis," said Alexander W. Charney, MD, Ph.D., co-senior author of the study and Associate Professor of Psychiatry, Genetic and Genomic Sciences, Neuroscience, and Neurosurgery at Icahn Mount Sinai and co-lead of the Living Brain Project.

"In doing so, we revealed more accurate insights into the prevalence and roles of A-to-I editing in the human brain. It is critical to note that our findings do not negate but instead provide missing context for using postmortem brain tissues in researching A-to-I regulation.

"Understanding these differences helps improve our knowledge of brain function and disease through the lens of RNA editing modifications, which can potentially lead to better diagnostic and therapeutic approaches."

The research team will further analyze the RNA editing data to understand its implications better and to identify potential therapeutic targets for Parkinson's disease. They are also expanding the research to include emerging work from this cohort that focuses on gene expression, proteomics, and multi-omics of the living brain.

"By harnessing the unique, transdisciplinary nature of the Living Brain Project, we can turn a cutting edge clinical care modality like deep brain stimulation into a platform for unprecedented insight into human brain biology that will give rise to new therapeutic opportunities," said Brian Kopell, MD, co-first author of the study, Director of the Center for Neuromodulation at Mount Sinai and co-lead of the Living Brain Project.

More information: Miguel Rodriguez de los Santos et al, Divergent landscapes of A-to-I editing in postmortem and living human brain, *Nature Communications* (2024). DOI: 10.1038/s41467-024-49268-z

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