Curing the incurable

RNA isoforms may hold the key to defeating Alzheimer's disease

Groundbreaking research on RNA isoforms in Alzheimer's disease (AD) has revealed another layer of genetic complexity that has been previously overlooked. Using cutting-edge sequencing technology, Dr. Mark Ebbert and colleagues at the Sanders-Brown Center on Aging at the University of Kentucky, USA, performed a detailed analysis of RNA isoforms in the human brain. They discovered multiple, previously unknown RNA isoforms from medically relevant genes, 245 entirely new genes, and differences between isoform expression in healthy and AD brains. These findings present promising targets for developing pre-symptomatic diagnostics and personalized therapies, offering hope to millions of people affected by currently incurable diseases.

Despite decades of research, our understanding of the underlying mechanisms driving Alzheimer's disease remains incomplete, hampering efforts to develop effective treatments and diagnostic tests. In fact, Alzheimer's disease is much more complex than it is often credited for and will ultimately require personalized therapies to truly defeat the disease. Alzheimer's disease exacts a heavy cost, not only on those directly affected but also on their families, caregivers, and society as a whole. While the need to develop meaningful and personalized therapies is widely recognized, no therapy can be truly meaningful without a pre-symptomatic diagnostic.

The remarkable human body can heal from basic physical injuries as though they never happened, but it's an entirely different story with Alzheimer's disease and other neurodegenerative diseases. By the time Alzheimer's disease symptoms onset, the disease has been ravaging the brain for potentially decades. Yet, because the brain is such an extraordinary organ, it can compensate for widespread neuronal death this entire time, until such excessive cell death has occurred that there simply isn't enough left for the brain to function properly. Thus, by the time symptoms have onset, it's too late for a 'meaningful' therapy to be truly meaningful. We need to identify the disease before symptoms onset, and preserve not only patient lives, but their memories – their relationships.

Alzheimer's disease causes excessive neuronal cell death.

While there is strong evidence that certain genetic factors contribute to disease risk, the precise mechanisms and molecular pathways involved in disease progression remain elusive. The Ebbert Lab hopes to change this. The team is working to identify how top Alzheimer's disease genes are actually involved in disease. A new pilot study by Dr. Mark Ebbert and colleagues from the Sanders-Brown Center on Aging at the University of Kentucky, USA, reveals a new perspective on gene function and diversity. Their findings further our understanding of the complex genetics underlying many human diseases, which may ultimately contribute to the development of new, personalized therapies and a pre-symptomatic diagnostic.

UNRAVELLING GENETIC COMPLEXITY

The researchers began by fundamentally challenging a common assumption that each human gene has a single function. A gene's function is realized by the production or 'transcription' of a messenger RNA (mRNA) molecule from the genomic DNA blueprint, followed by 'translation' of the mRNA into a protein, which can carry out its function in the body. Dr. Ebbert likes to think of proteins as little workers in the body, performing a wide range of jobs that make life possible. This picture gets far more complicated as it becomes clear the human genome is not a static blueprint but a dynamic script, with multiple modifications occurring in response to internal molecular signals, external environmental challenges, and disease states.

One form of these modifications is called 'alternative splicing' – a process of mRNA editing that occurs after transcription. Alternative splicing results in different mRNA molecules (called 'isoforms') being produced from the same gene that are then translated into distinct proteins (i.e., workers). It is estimated that in the human genome, each protein-coding gene averages over eight mRNA isoforms. The importance of this complexity cannot be overstated, as some isoforms from the same gene are known to play different or even opposite roles within a cell, demonstrating not all proteins from a single gene perform the same function. Additionally, previous work demonstrated that the distinct proteins from a single gene often have entirely unique protein interactions in the cell. Ebbert explains: 'Assuming that most isoforms are noise may be akin to assuming alternative splicing is simply a biological accident rather than an evolutionarily derived process that enables biological diversity and complexity.'

To develop meaningful therapies, we need to identify Alzheimer's disease before symptoms onset.

Because directly studying proteins remains challenging, researchers typically study the RNA and then make inferences about protein production and activity using this data. The most common technology currently employed to study RNA is called 'short-read sequencing'. As the name implies, this method involves reading short sections of the RNA molecules and piecing them together to construct a picture of the RNAs present, along with an estimate of their quantity. A major limitation of this technology, however, is that researchers are forced to collapse all isoforms into a single RNA measurement for that gene – resulting in a major over-simplification of the underlying biology.

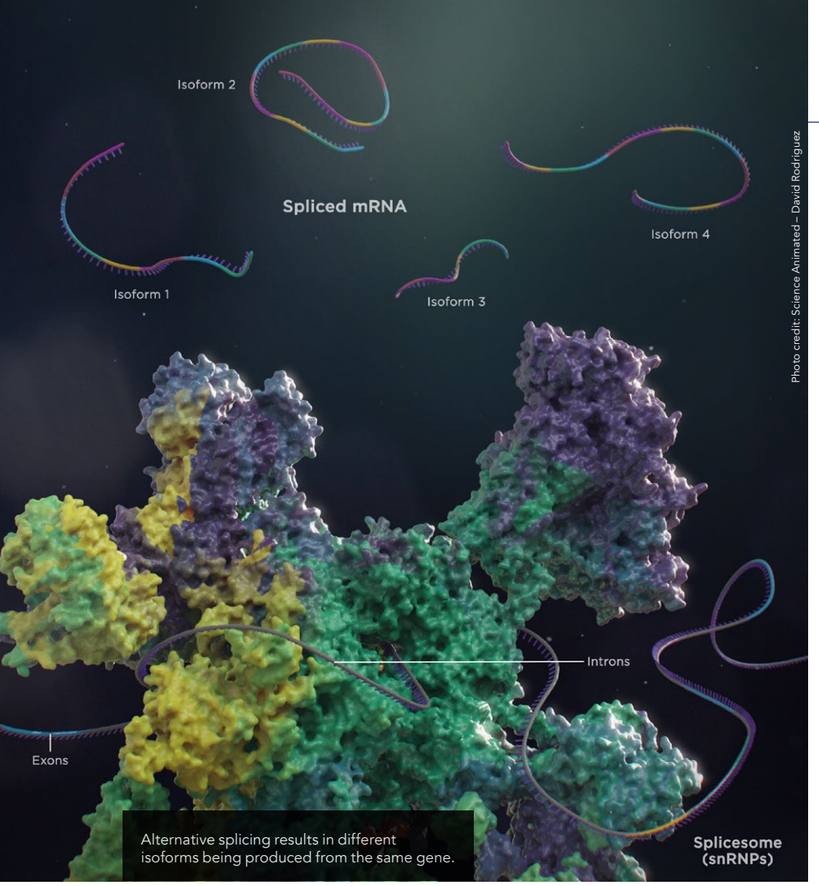
NEW TECHNOLOGY, NEW INSIGHTS

To address this limitation, Ebbert and his team used an alternative, cutting-edge technology called 'long-read sequencing', which makes it possible to characterize and quantify RNA isoforms directly. Accurately measuring all RNA isoforms produced from a gene makes it possible to finally determine the individual function for each isoform. Further, it can lead to discovering previously unknown isoforms, which could improve our understanding of disease mechanisms and potentially provide new therapeutic targets for disease diagnosis and treatment. This is especially important for complex diseases lacking effective therapies, such as Alzheimer's disease and certain cancers.

For this reason, Ebbert's team focused their research on post-mortem brain tissue samples obtained from individuals diagnosed with Alzheimer's disease and compared them with samples from individuals without neurological disease. The deep long-read sequencing they performed yielded an average of 35.5 million aligned reads per sample, compared to previous studies, which averaged 6 million or fewer aligned reads per sample. With this deep long-read sequencing data, the Ebbert Lab discovered 245 entirely new genes and over 700 previously undescribed RNA isoforms. Out of the 700, 53 were from 'medically relevant' genes – genes associated with a range of human diseases, including neurological disorders. The functions of these genes ranged from involvement in neuronal function and synaptic transmission to neuroinflammation, highlighting the complexity of these disorders.

The team also made some unexpected discoveries, including discovering new RNA isoforms originating from mitochondrially encoded genes – the sub-cellular organelles responsible for generating energy in every cell of the body. This discovery represents a previously unrecognized level of complexity in mitochondrial RNA processing. Given the crucial role of mitochondria in cellular energy metabolism and their implication in neurodegenerative disorders, this finding opens an exciting new avenue of exploration for potential mechanisms underlying diseases, including Alzheimer's disease, where researchers believe mitochondrial dysfunction is involved.

The team's findings further our understanding of the complex genetics underlying many human diseases, which may ultimately contribute to the development of new, personalized therapies and a pre-symptomatic diagnostic for Alzheimer's disease.



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Being able to accurately quantify RNA isoforms will be essential to understanding the effects of genetic variations in multiple diseases.

When comparing samples from Alzheimer's disease brains to healthy controls, significant differences in both the genes being expressed and RNA isoform abundance from those genes were uncovered. In this preliminary study, the researchers identified 99 RNA isoforms that were either up- or down-regulated in Alzheimer's disease patient brains compared to controls (i.e., differentially expressed), highlighting significant gaps in our understanding of gene expression and its implications for disease. Arguably most importantly, some differentially expressed isoforms originated from genes that were not differentially expressed at the gene level (i.e., when collapsing all isoforms from the given gene into a single RNA measurement) and, therefore, would have flown completely under the radar in previous studies relying on short-read sequencing technologies.

This underscores the need for long-read sequencing and isoform-specific analyses, emphasizing the importance of considering isoform diversity in disease diagnosis and treatment.

A PARADIGM SHIFT IN PERSONALIZED MEDICINE?

While the implications of this new technology are profound, this study represents only the first step on a long road to improving our understanding of genetic diversity and function in health and disease. The Ebbert Lab's findings are important, but as Dr. Ebbert says, 'It is not enough. There is so much more to do.' Long-read sequencing will be an essential technology for the future of the field of functional genomics, but the benefits extend far beyond the lab. Being able to accurately quantify RNA isoforms will be essential to understanding the effects of genetic variations in multiple diseases, revealing disease-associated patterns that are undetectable at the genomic level. These insights will, in turn, offer new opportunities to develop personalized therapies and pre-symptomatic diagnostics.

Novel pre-symptomatic diagnostic tools will be particularly important in diseases such as Alzheimer's disease. By the time symptoms of the condition are noticeable, the neuronal loss and damage caused to the brain have been underway for years, potentially decades, and even exceptionally efficacious therapies would have limited impact. Identifying patients requiring treatment for these conditions before irreparable damage is done is vital to achieving improved outcomes.

Treating these complex diseases may require advances in 'personalized medicine', also known as precision medicine. This is an approach to healthcare that tailors medical treatment and interventions to individual patient characteristics, including their genetic makeup, environmental factors, and lifestyle. As Dr. Ebbert and colleagues show in their study, however, some differences in RNA isoform abundance are not reflected in traditional gene expression measurements. Subtle differences in RNA isoform expression may explain why some patients respond to current drugs and treatments while others do not, highlighting the need for alternative therapies for patients presenting with different isoform expression patterns.

Long-read sequencing represents a significant improvement in our understanding of human genetics and will inevitably shed light on the complex mechanisms that govern human health and disease. As scientists such as Dr. Ebbert and his colleagues continue to explore the previously hidden world of RNA isoforms, new therapeutic targets will inevitably be uncovered, novel diagnostic tests developed, and revolutionary advancements in personalized medicine achieved – paving the way to a future where currently incurable diseases such as Alzheimer's disease are not just treatable, they're forgotten, transforming millions of lives and reshaping the future of healthcare.

Behind the Research

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Research Objectives

The Ebbert Lab studies the mechanisms driving Alzheimer's disease to help develop personalized disease therapies and a pre-symptomatic disease diagnostic.

Detail

Bio

Dr. Mark Ebbert is an Assistant Professor at the University of Kentucky's Sanders-Brown Center on Aging. He has a background in computational biology and bioinformatics, focusing on human genomics and its application to Alzheimer's disease. His lab's focus is to help develop personalized disease therapies and a pre-symptomatic disease diagnostic.

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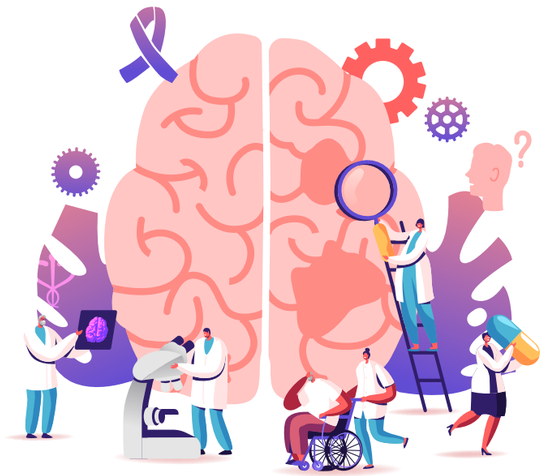

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Personal Response

What time frame do you anticipate for the incorporation of RNA isoform analysis into medical diagnosis, drug development, and clinical treatment?

"This is an exciting time in research where we are finally beginning to give proper attention to distinct RNA and protein isoforms arising from a 'single' gene. One of our primary goals in the Ebbert Lab is to push RNA and protein isoform analysis directly into diagnosis, drug development, and clinical treatment as quickly as possible. It's very ambitious, but I hope to see the first fruits within 5 to 10 years."

What are the key barriers to implementing RNA isoform analysis in precision medicine initiatives in clinical practice?

"Excellent question. There is simply such a large gap in our understanding of the different RNA isoforms. Scientists have known about alternative splicing (i.e., distinct isoforms arising from a single gene) for approximately 50 years, but they have largely been ignored because studying them via high-throughput means has not been possible. We can finally do that, so we can finally start to understand what job each isoform is performing and how it differs from others originating from the same gene. I believe we will begin to understand many of the subtleties of human health and disease at a level never before possible because of these technologies."

Sanders-Brown Center on Aging