Combining long- and short-read sequencing in single cells reveals new mRNAs in neurodegenerative diseases

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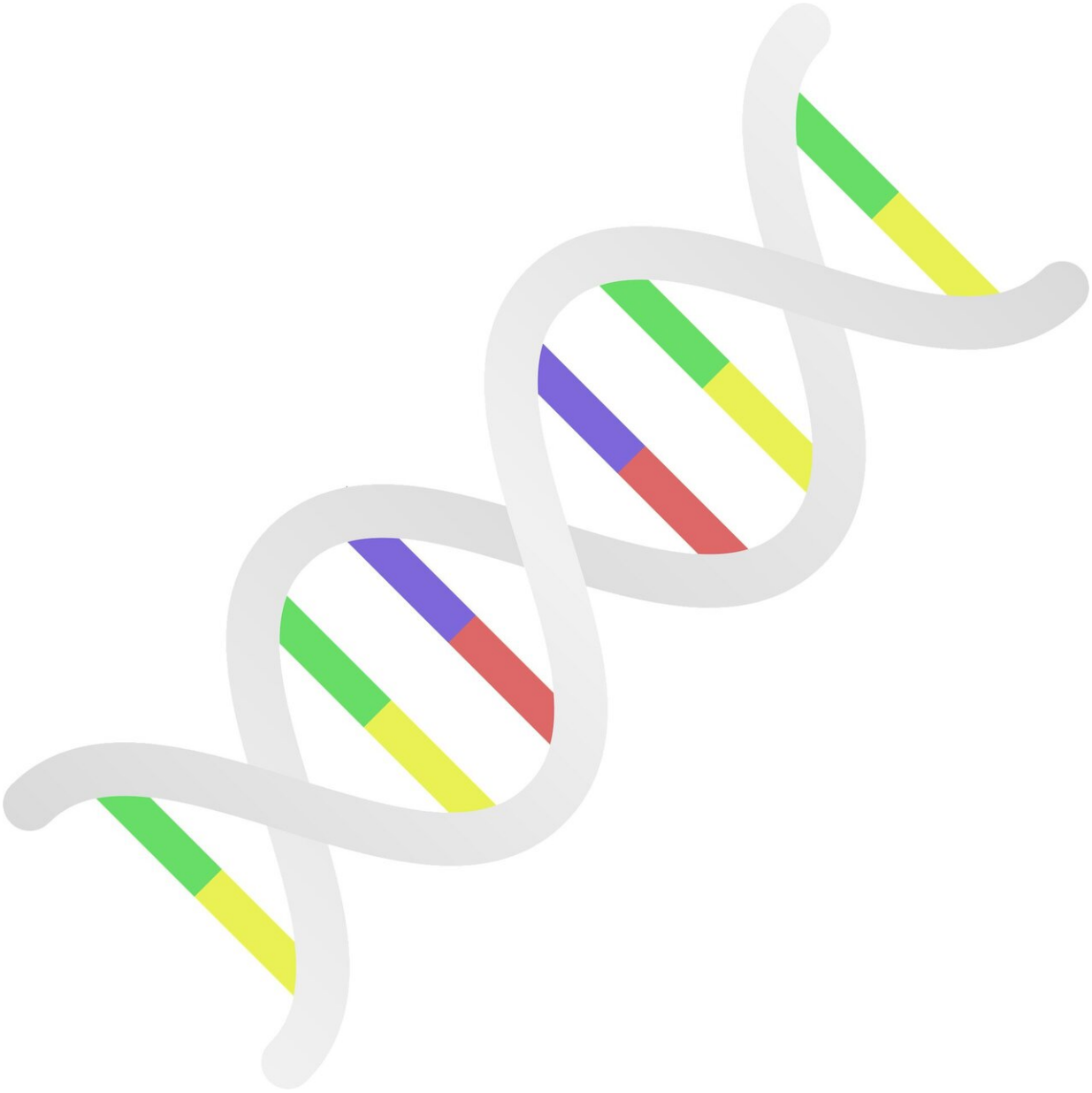

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Diseases marked by progressive deterioration of the brain—neurodegeneration—have proven challenging to understand and treat. These common conditions affect millions of patients and families around the world, yet efforts to develop new therapies have largely been unsuccessful.

Scientists at Sanford Burnham Prebys are uncovering new ideas for future treatments by better understanding the effects of neurodegenerative diseases on our brain cells.

Researchers led by Jerold Chun MD, Ph.D., professor in the Degenerative Diseases Program at Sanford Burnham Prebys, have published results in *eNeuro* from combining two sequencing technologies in single cells to find new differences in mRNAs resulting from Alzheimer's disease (AD), dementia with Lewy bodies (DLB) and Parkinson's disease (PD). Genes can produce more than one messenger RNA (mRNA)—and, thus, more than one protein—through a process known as alternative splicing. These different mRNAs are called isoforms.

The team used two forms of single-nucleus RNA sequencing (snRNAseq) in the study, which followed up on the landmark report in *Science* in 2016 by Chun and his collaborators about the use of snRNAseq in the human brain.

"Now, snRNAseq is the gold standard for looking at single cell transcriptomes in the human brain," said Chun. "Because of the brain's

complex intermixture of cells that can have thousands of connections, other single-cell technologies are more apt to be contaminated by the stuff around the cell that you don't want."

Using snRNAseq avoids that dilemma by isolating the nuclei of each cell in a sample. Then, scientists can analyze the composition of RNA molecules that contain codes for building new proteins.

"However, typical single-cell sequencing experiments use what is called short-read sequencing," said Christine Liu, Ph.D., a postdoctoral associate in the Chun lab and the study's first author. "This method reads 100 to 150 base pairs at a time and compares each to a reference genome."

These comparisons are used to map the smaller sequences to a reference sequence. Differences from the reference genome are what scientists call variants. Using short-read snippets to reconstruct the whole, however, has limitations.

"Short-read sequencing struggles with certain kinds of sequence variants, so to better capture these, we also used long-read sequencing that reads between 5,000 and 30,000 base pairs at a time and does not require mapping to a reference genome," said Chun.

The research team applied both techniques to single cells from post-mortem brain tissue samples from 25 donors who suffered from either AD, DLB or PD, as well as to samples from donor brains without neurodegenerative diseases, which served as the experiment's control group. In their assessment of more than 165,000 cells, the group used targeted long-read sequencing of mRNAs for the 50 genes most associated with the three neurodegenerative diseases in prior research.

The findings included uncovering new mRNA sequences from all 50

target genes that had not been found by previous sequencing experiments.

"By combining short- and long-read sequencing, we found vast mRNA isoform diversity in these genes, even those that were not differentially expressed in the short-read data," said Liu. "In some of the genes, the novel transcripts we identified actually seem to be a majority of the total isoforms."

"Our results reinforce our previous findings that three-quarters of the mRNAs in the brain transcriptome were unknown," said Chun, referring to a 2021 *PNAS* paper that reported finding hundreds of thousands of new mRNA transcripts. "We still have a lot left to learn about these new mRNAs and how they change with disease."

Another question for the research team is what types of novel proteins are being produced from these transcripts.

"New mRNA isoforms mean new potential proteins within diseased brains and cells," said Chun, "which might represent something previously invisible that can now be therapeutically targeted to find treatments for these common and debilitating diseases."

Additional authors on the study from Sanford Burnham Prebys include Chris Park, Tony Ngo, Janani Saikumar, Carter R. Palmer, Anis Shahnaee and William J. Romanow.

More information: Christine S. Liu et al, RNA isoform diversity in human neurodegenerative diseases, *eneuro* (2024). DOI: 10.1523/ENEURO.0296-24.2024

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