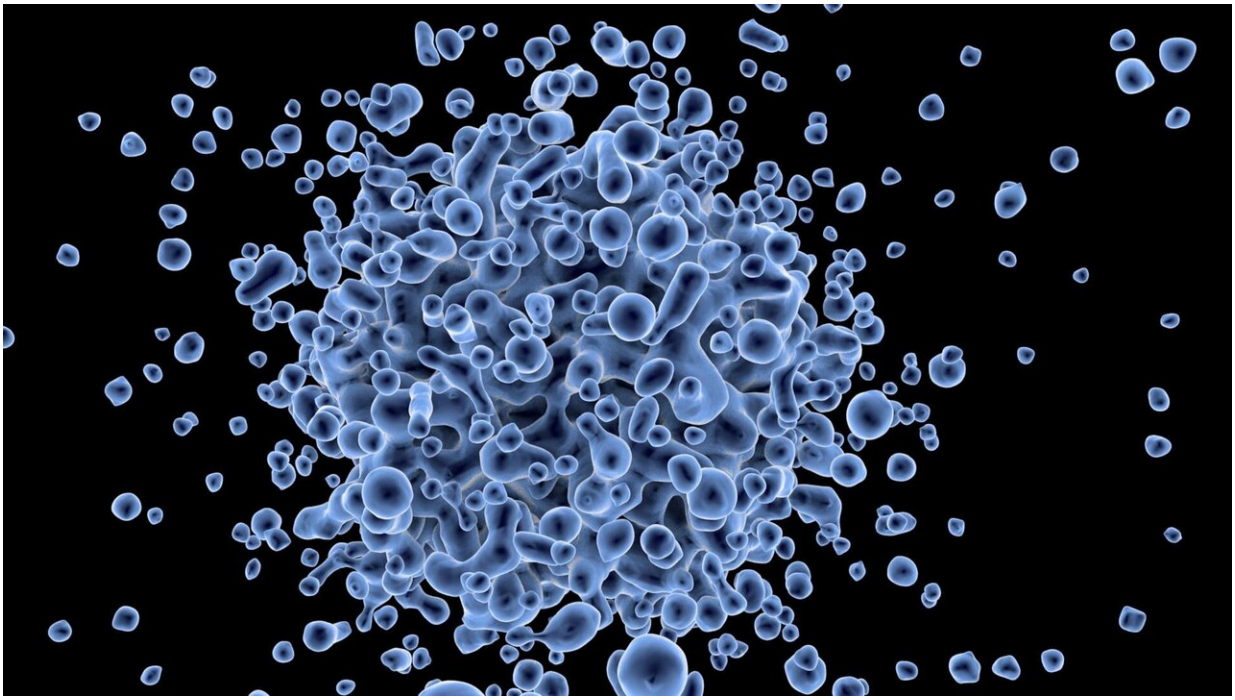
'Silent' mutations found to have repercussions beyond their own gene

August 28 2024, by Brandi Wampler



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Genetic disorders—like cystic fibrosis and Huntington's disease—are considered incurable, with gene mutations occurring in essentially every cell of the body. Gene mutations occur when one nucleotide in a codon is switched. In non-synonymous mutations, this disrupts the codon's function to code for its amino acid. In synonymous mutations, the codon still codes the correct amino acid. As such, these mutations are dubbed

"silent" and often considered inconsequential to human health.

Now, researchers from the University of Notre Dame are adding new evidence to the emerging concept that these silent mutations may have crucial consequences. Their study, published in the *Proceedings of the National Academy of Sciences*, showed how a synonymous mutation in one gene can significantly affect a neighboring gene, increasing its protein production.

"The dogma in the field right now is that within the protein coding part of the genome, the only mutations that matter are the ones that change the DNA to code from one amino acid to another," said Patricia L. Clark, the O'Hara Professor of Chemistry and Biochemistry at Notre Dame and lead author of the study. "That's a very oversimplified view—to the point of being detrimental—of what matters."

For this study, researchers experimented with the genome of the bacteria E. coli, as its small genome and simple cell structure make it more straightforward to ask fundamental questions about the impact of mutations than human cells. They created nine different synonymous versions of the CAT (Chloramphenicol acetyltransferase) gene, with each using different synonymous codons to encode the CAT protein.

When those different synonymous versions were expressed, they discovered that four of nine synonymous sequences affected the number of CAT proteins synthesized.

"Think about synonymous mutations like a huge quilt of possible DNA sequences that are all going to give you the same protein," Clark said. "You can pick any part of the quilt and get the same protein, but will you get the same amount of protein? Will the protein fold be the same? Is the cell going to be healthy? This is what we were looking at."

As an expert in protein folding, Clark formed the initial hypothesis that these four synonymous mutations might be altering CAT protein folding, which occurs after gene expression. However, the researchers—including first author Anabel Rodriguez, then a doctoral student in Clark's lab—went on to discover that the impact of the synonymous mutations occurs during the gene expression process, affecting the transcription of DNA to RNA.

"What Anabel showed was that the amount of CAT protein synthesis was correlated to the amount of CAT RNA synthesis," Clark said. "This indicated that some synonymous mutations screwed up the synthesis of RNA from DNA. That Anabel was able to figure out this novel transcriptional regulation mechanism, while working in a lab with no previous experience studying transcription, is a remarkable achievement."

The research showed that some of the synonymous mutations created cryptic transcription sites on the CAT DNA strand. RNA polymerase, the enzyme responsible for transcribing DNA to RNA, was binding to these cryptic transcription sites—instead of their expected binding site.

These polymerases synthesized an RNA that started within CAT, but extended to also encode the entire neighboring, upstream gene. In the case of CAT, the upstream gene encodes a repressor protein, so making more of it represses the expression of CAT.

The concept of a synonymous mutation impacting its own gene's processes has only been considered in the last decade. So the idea that a synonymous mutation on one gene could also affect the transcription and translation processes of a neighboring gene is a significant expansion—and something Clark and her lab plan to further explore.

"There has been an increasing number of landmark studies that show

how incomplete our understanding is on the impact of synonymous mutations. We should be considering how these mutations impact all diseases and genetic disorders," Clark said. "I hope that our study will help accelerate the building of a comprehensive understanding."

Next, the research team plans to analyze how some of the synonymous mutations of the CAT gene were able to recruit RNA polymerase to the cryptic binding location so efficiently. This is especially intriguing given that the currently available machine learning algorithms have not been able to accurately predict it.

Other study co-authors include Jacob Diehl, Christopher Bonar, Taylor Lundgren, McKenze Moss, Jun Li, Tijana Milenkovic, Paul Huber and Matthew Champion from Notre Dame; Gabriel Wright from the Milwaukee School of Engineering; and Scott Emrich from the University of Tennessee.

More information: Anabel Rodriguez et al, Synonymous codon substitutions modulate transcription and translation of a divergent upstream gene by modulating antisense RNA production, *Proceedings of the National Academy of Sciences* (2024). DOI: 10.1073/pnas.240551012

Provided by University of Notre Dame