Most 'silent' genetic mutations are harmful, not neutral, a finding with broad implications

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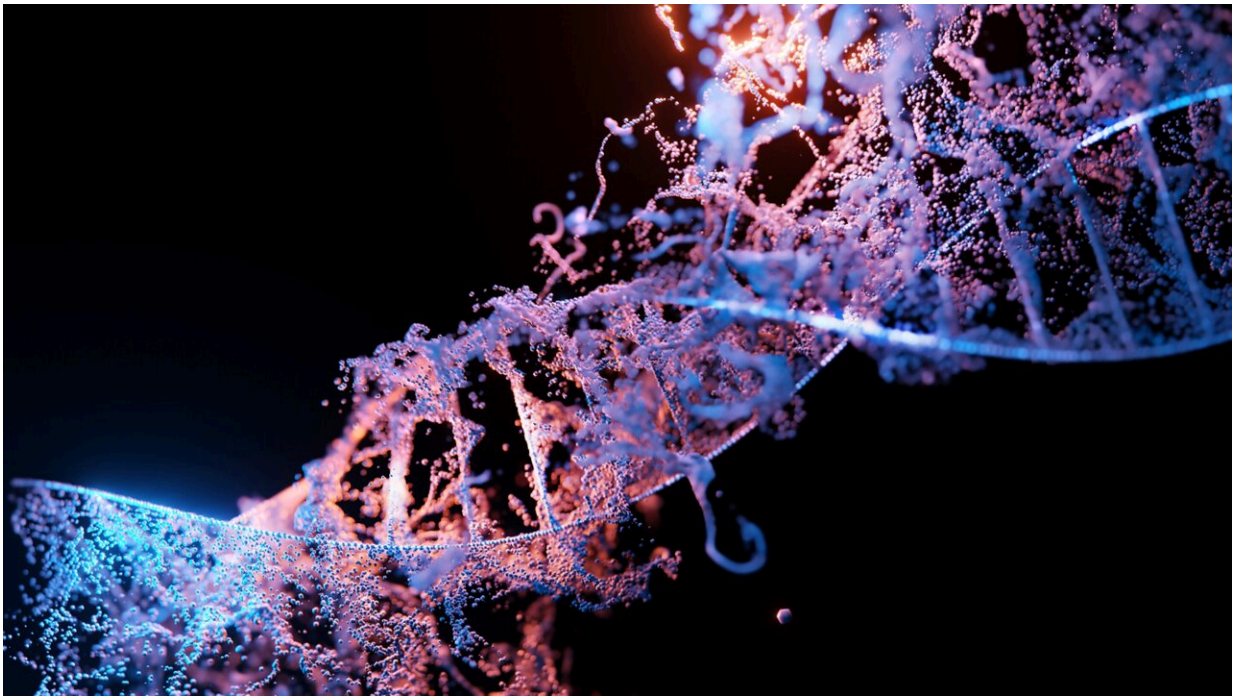

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In the early 1960s, University of Michigan alumnus Marshall Nirenberg and a few other scientists deciphered the genetic code of life, determining the rules by which information in DNA molecules is translated into proteins, the working parts of living cells.

They identified three-letter units in DNA sequences, known as codons,

that specify each of the 20 amino acids that make up proteins, work for which Nirenberg later shared a Nobel Prize with two others.

Occasionally, single-letter misspellings in the genetic code, known as point mutations, occur. Point mutations that alter the resulting protein sequences are called nonsynonymous mutations, while those that do not alter protein sequences are called silent or synonymous mutations.

Between one-quarter and one-third of point mutations in protein-coding DNA sequences are synonymous. Ever since the genetic code was cracked, those mutations have generally been assumed to be neutral, or nearly so.

But in a study scheduled for online publication June 8 in the journal *Nature* that involved the genetic manipulation of yeast cells in the laboratory, University of Michigan biologists show that most synonymous mutations are strongly harmful.

The strong non-neutrality of most synonymous mutations—if found to be true for other genes and in other organisms—would have major implications for the study of human disease mechanisms, population and conservation biology, and evolutionary biology, according to the study authors.

"Since the genetic code was solved in the 1960s, synonymous mutations have been generally thought to be benign. We now show that this belief is false," said study senior author Jianzhi "George" Zhang, the Marshall W. Nirenberg Collegiate Professor in the U-M Department of Ecology and Evolutionary Biology.

"Because many biological conclusions rely on the presumption that synonymous mutations are neutral, its invalidation has broad implications. For example, synonymous mutations are generally ignored

in the study of disease-causing mutations, but they might be an underappreciated and common mechanism."

In the past decade, anecdotal evidence has suggested that some synonymous mutations are nonneutral. Zhang and his colleagues wanted to know if such cases are the exception or the rule.

They chose to address this question in budding yeast (Saccharomyces cerevisiae) because the organism's short generation time (about 80 minutes) and small size allowed them to measure the effects of a large number of synonymous mutations relatively quickly, precisely and conveniently.

They used CRISPR/Cas9 genome editing to construct more than 8,000 mutant yeast strains, each carrying a synonymous, nonsynonymous or nonsense mutation in one of 21 genes the researchers targeted.

Then they quantified the "fitness" of each mutant strain by measuring how quickly it reproduced relative to the nonmutant strain. Darwinian fitness, simply put, refers to the number of offspring an individual has. In this case, measuring the reproductive rates of the yeast strains showed whether the mutations were beneficial, harmful or neutral.

To their surprise, the researchers found that 75.9% of synonymous mutations were significantly deleterious, while 1.3% were significantly beneficial.

"The previous anecdotes of nonneutral synonymous mutations turned out to be the tip of the iceberg," said study lead author Xukang Shen, a graduate student research assistant in Zhang's lab.

"We also studied the mechanisms through which synonymous mutations affect fitness and found that at least one reason is that both synonymous

and nonsynonymous mutations alter the gene-expression level, and the extent of this expression effect predicts the fitness effect."

Zhang said the researchers knew beforehand, based on the anecdotal reports, that some synonymous mutations would likely turn out to be nonneutral.

"But we were shocked by the large number of such mutations," he said. "Our results imply that synonymous mutations are nearly as important as nonsynonymous mutations in causing disease and call for strengthened effort in predicting and identifying pathogenic synonymous mutations."

The U-M-led team said that while there is no particular reason why their results would be restricted to yeast, confirmations in diverse organisms are required to verify the generality of their findings.

The other authors of the *Nature* study are Siliang Song of the U-M Department of Ecology and Evolutionary Biology and Chuan Li of Stanford University.

More information: Jianzhi Zhang, Synonymous mutations in representative yeast genes are mostly strongly nonneutral, *Nature* (2022). DOI: 10.1038/s41586-022-04823-w.
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