Scientists discover mechanistic link between genetic variation and risk of cardiovascular disease

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A team from UNC Lineberger Comprehensive Cancer Center has uncovered a clue as to how certain common genetic variants may influence an individual's risk of developing cardiovascular diseases such as stroke or heart attack.

The team had been studying three related genes encoded by the INK4/ARF (or CDKN2a/b) locus which is closely associated with human aging. Previous genome-wide association studies (GWAS) from several large consortia have shown that common genetic variants located very near, but not actually in, these genes are associated with diseases of aging such as cancer, heart attack, stroke, type 2 diabetes and frailty. Yet how these variants contribute to the risk of these common human ailments was not known.

The UNC team, led by post-doctoral fellow Christin Burd, PhD, and supervised by UNC Lineberger associate director Ned Sharpless, MD, thought a piece of cellular machinery called a long non-coding RNA might be involved. Long non-coding RNAs are RNA molecules that are not translated into proteins, but which appear to regulate the expression of nearby genes. The number of these molecules encoded in the human genome is unknown, but thought to be in the thousands. However, since the study of these molecules is a new field, the vast majority of these RNAs are of unknown function.

In the course of investigating the link between the aging-related INK4/ARF variants and an associated long non-coding RNA, the team came across a finding so unexpected that, according to Sharpless, they thought it was a simple laboratory mistake for several months, despite multiple repetitions. The group kept observing that expressed non-coding RNA sequences linked to the INK4/ARF locus occurred in what appeared to be the "wrong order". They showed that these mis-ordered RNAs were not a mistake or byproduct of the experimental methodology, demonstrating that blood samples from more than 100 different individuals all contained these unexpected RNA forms. They considered several explanations for the seemingly incorrect sequence of these RNAs, but eventually proved these data represented the unexpected production of circular RNAs. Circular RNAs are very rare in humans, having been viewed as a biological curiosity without known function.

"Outside a limited set of viral genomes, circular RNAs have not frequently been observed in nature, and less than one percent of all human genes are even predicted to behave this way," said Burd.

The circular forms are thought to arise through specialized splicing of a long RNA that represses the proteins encoded by the INK4/ARF locus. Although all individuals appear to produce circular RNAs associated with the INK4/ARF locus, some individuals make relatively more of these forms than others. The group showed that individuals who produce more of the circular form of this long non-coding RNA exhibit increased expression of INK4/ARF genes. Sharpless' lab and others have previously shown that expression of INK4/ARF genes strongly affects human susceptibility to certain cancers and diseases of aging, such as atherosclerosis. Therefore, these new findings suggest that common genetic variants within the non-coding RNA influence a person's propensity to produce these circular forms which in turn controls INK4/ARF expression and risk of aging-associated diseases such as

atherosclerosis.

"We believe that people carrying the atherosclerosis-associated genetic variants splice the long RNAs less efficiently and make fewer circular RNAs. As a result, they also have lower levels of the INK4/ARF gene products, which usually limit abnormal proliferation of the cells that form atherosclerotic plaques." Burd noted.

"We still don't know exactly how this works," said Sharpless, "but it is the first time that one of these odd circular RNAs has been linked to an actual human disease. This finding that we thought was an error appears to serve an important function in controlling cellular proliferation. We showed this correlates with risk of heart attack and stroke, but believe this finding has significance for several other common diseases associated with human aging."

More information: The finding was published today in the open-access journal *PLoS Genetics*.

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