Unraveling the roles of non-coding DNA explains childhood cancer's resistance to chemotherapy

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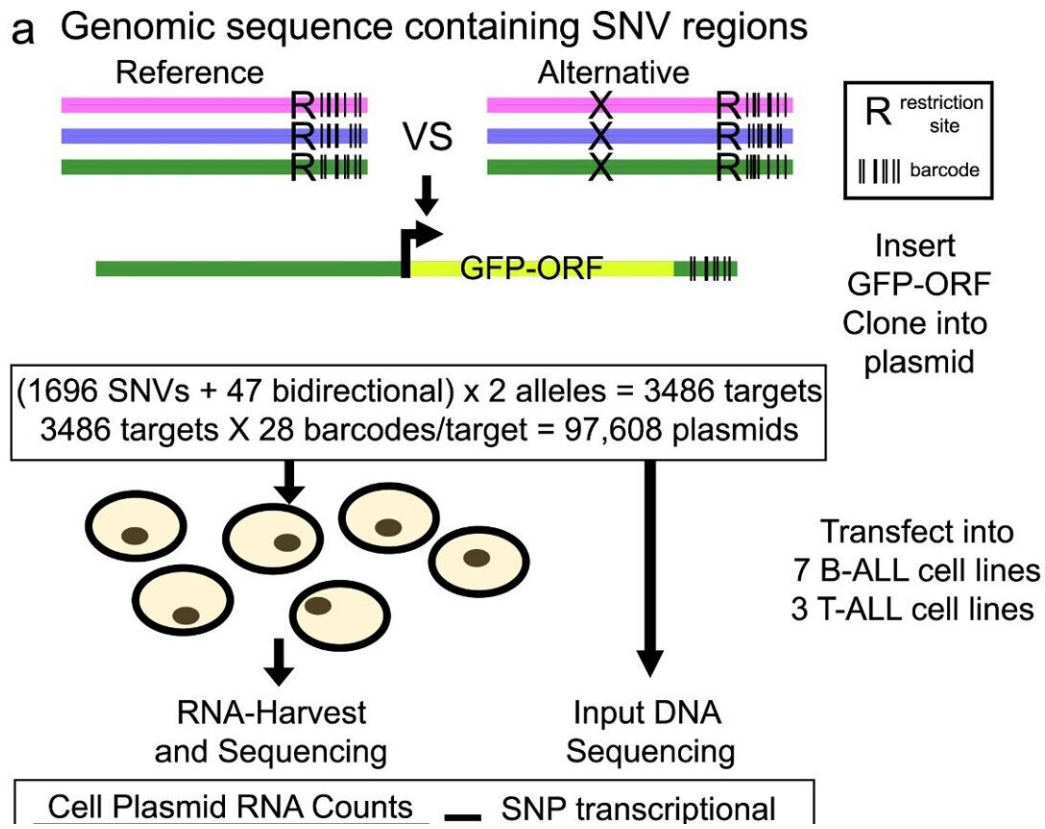


MPRA identifies regulatory variants with allele-specific effects on gene expression. Credit: *Nature Communications* (2024). DOI: 10.1038/s41467-024-48124-4

St. Jude Children's Research Hospital scientists have identified specific DNA variants in the non-coding regions of the genome contributing to chemotherapy resistance in acute lymphoblastic leukemia (ALL). The results guided the team to unravel the mechanism behind a previously unknown contributor to therapeutic resistance. The discovery was enabled by combining new technologies to overcome previous limitations in understanding the non-coding genome, which could be adapted to other types of cancer and diseases.

The findings are published in *Nature Communications*.

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Survival rates are over 94% due to modern therapy. However, those with relapsed or recurrent disease, often due to chemotherapy resistance, have a much poorer 30–40% survival rate.

The researchers studied resistance variants found in the non-coding genome, which makes up 98% of DNA and does not contain genes. Previous attempts to identify resistance mechanisms to chemotherapy had focused on DNA that encoded genes. Looking directly at genes is simpler because non-coding DNA can have complex relationships with gene function, but the St. Jude group has shown that it is possible.

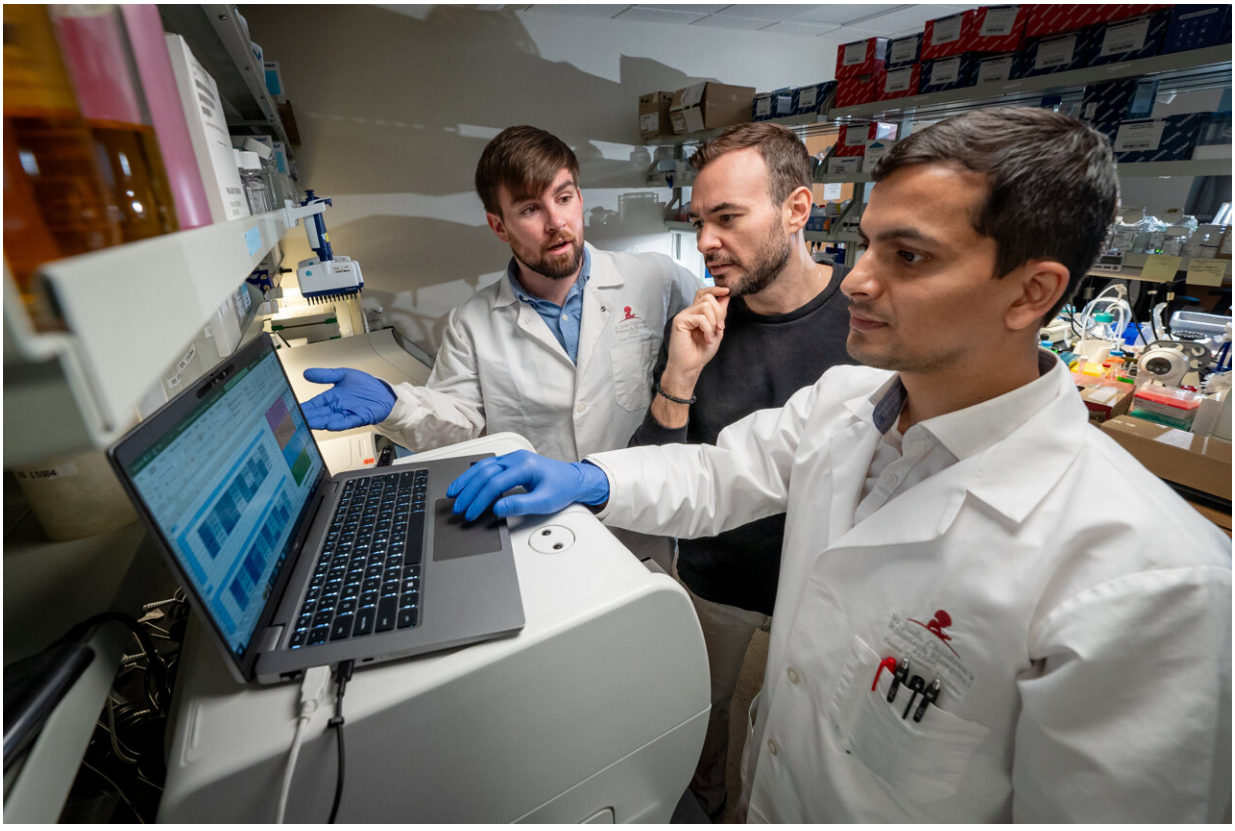
"We demonstrated that we now have the tools to find relevant non-coding genetic factors that contribute to chemotherapy resistance," said corresponding author Daniel Savic, Ph.D., St. Jude Department of Pharmacy and Pharmaceutical Sciences. "The end goal is to understand the mechanisms of drug resistance so we can develop novel therapeutics and optimize existing chemotherapies based on the individual's unique genetic makeup."

Sorting through non-coding DNA to find the root of

chemotherapy resistance

"The non-coding 98% of the genome contains instructions," said co-first author Jackson Mobley, Ph.D., St. Jude Department of Pharmacy and Pharmaceutical Sciences. "If we are making a building, genes encode the iron bars, wires and concrete; non-coding DNA are the blueprints. We found the small changes in the blueprints that impact how well you respond to certain therapies."

The group explored novel non-coding resistance variants by combining state-of-the-art technologies to examine patient samples and clinical data on treatment outcomes. In the past, research had focused on a single gene or variant. However, combining high-throughput DNA sequencing methods allowed the St. Jude researchers to perform massively parallel variant screens.



(L to R) Co-first author Jackson Mobley, Ph.D., corresponding author Daniel Savic, Ph.D., and co-first author Kashi Raj Bhattarai, Ph.D., all of the St. Jude Department of Pharmacy and Pharmaceutical Sciences. Credit: St. Jude Children's Research Hospital

Those large screens enabled the testing of over 1,600 variants simultaneously to identify which were functional. That huge increase made the results more comprehensive, leading to the discovery of over 500 functional non-coding DNA variants associated with chemotherapy resistance.

"Our work represents the largest functional investigation of inherited non-coding variants associated with pharmacological traits, especially in ALL," said co-first author Kashi Raj Bhattarai, Ph.D., St. Jude Department of Pharmacy and Pharmaceutical Sciences. "We verified that identified variants also have a similar effect in cell lines and patient samples."

A novel resistance mechanism

By surveying many non-coding variants at once, the researchers were able to find the most impactful ones across different subtypes of ALL and connect them to a specific gene using innovative 3D genome mapping technologies. By finding the mechanism behind how variants in the non-coding genome affect target gene activity, they can figure out how it affects cancer's response to treatment.

For example, the top variant from the screen led to the discovery of a new resistance mechanism. The resistance was to the chemotherapy drug

vincristine. The researchers examined how DNA containing the functional variant physically looped to its target gene and which transcription factors, proteins that guide gene expression, were involved.

The scientists found the variant bound near the gene for EIF3A, which is known to be involved in cell proliferation and survival. When they deleted the DNA containing the variant or reverted the mutation to the original sequence, they could alter the cells' sensitivity to the chemotherapeutic agent vincristine.

The study serves as a proof of principle of how to take non-coding DNA variants and mechanistically connect them to a trait, such as chemotherapy resistance. That has been a long-standing issue holding back genomics research on inherited variants, from cancer to neurological issues.

"In any genome-wide association study, nearly all associated variants reside in the non-coding genome," Savic said. "Therefore, connecting that variation to gene function and then to an actual trait, such as chemotherapy resistance or disease predisposition, is challenging. We showed that we have harnessed tools and technologies to systematically examine the non-coding genome and understand what it's doing. We hope that our findings can be utilized to improve clinical outcomes in ALL patients."

More information: Kashi Raj Bhattarai et al, Investigation of inherited noncoding genetic variation impacting the pharmacogenomics of childhood acute lymphoblastic leukemia treatment, *Nature Communications* (2024). DOI: 10.1038/s41467-024-48124-4

Provided by St. Jude Children's Research Hospital