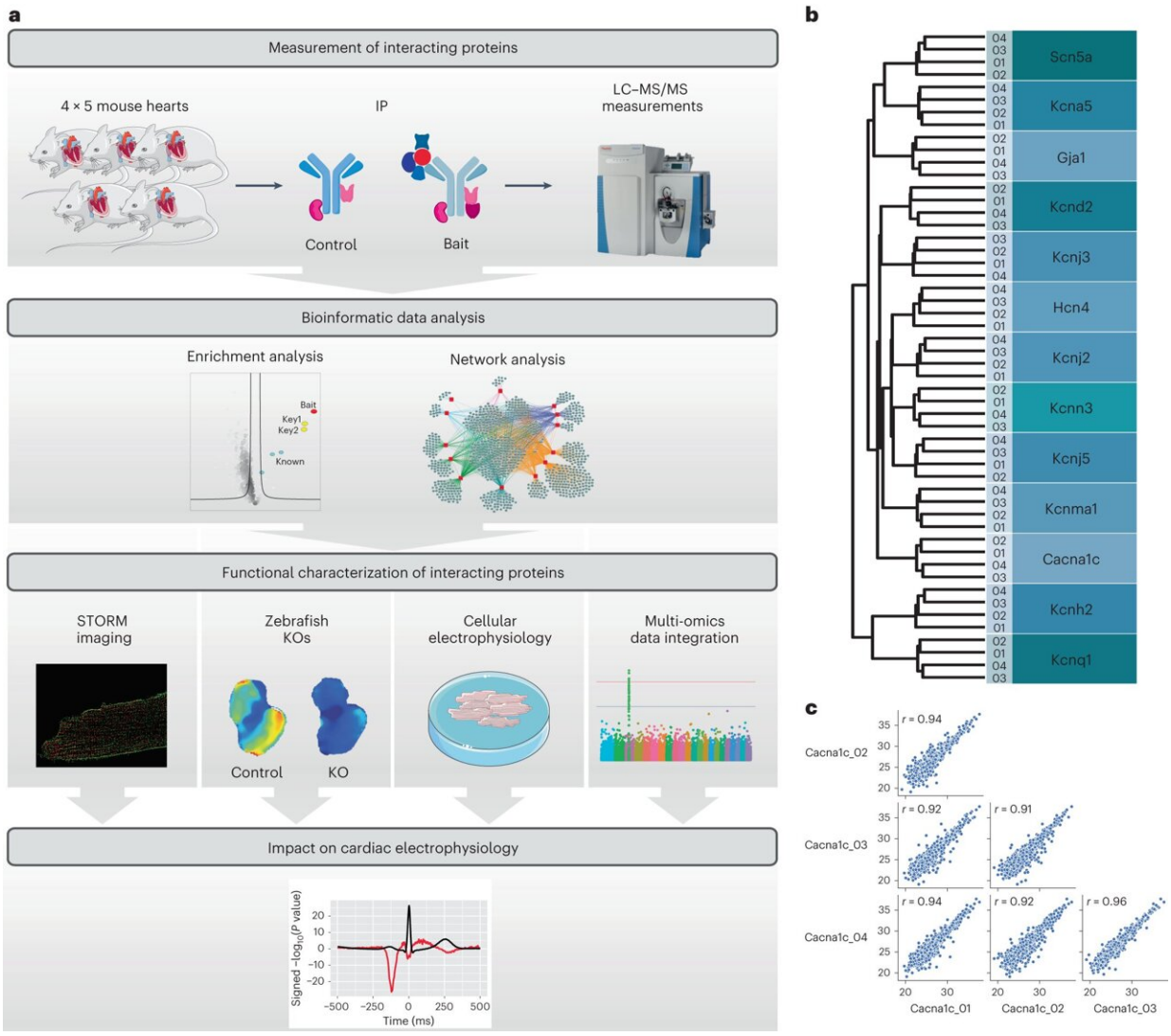
Researchers find heartbeat relies on surprisingly large network of proteins

August 16 2023



MS evaluation of cardiac ion channel IPs. a, Workflow of the study. We performed MS measurements of immunoprecipitated channels and their interactors and of control IPs from quadruplicate murine cardiac tissue lysates.

Deep proteome measurements of the membrane-enriched mouse heart samples utilized in the IP experiments were also performed. Bioinformatics network analyses prioritized interactors for functional evaluation. A subset of interactors were evaluated for their functional impact on cardiac electrophysiology by STORM imaging, optical mapping in zebrafish KOs, and patch clamping of cardiomyocytes from mice with interactor genes silenced. From multi-omics data integration, the impact of each interactor in human electrophysiology is evaluated. b, Dendrogram from unsupervised hierarchical cluster analysis of protein intensities of proteins identified in IP experiments show that the four replicate experiments all cluster together. The clustering follows the bait replicates. c, Pearson correlation coefficients for protein intensities of the four Cacna1c replicate pulldown experiments. Pearson correlation coefficients are indicated in each scatter plot. Credit: *Nature Cardiovascular Research* (2023). DOI: 10.1038/s44161-023-00294-y

The first mapping of the heart's crucial ion channels reveals a surprisingly extensive network of proteins. This understanding is the first step towards more precise treatment for patients with cardiac arrhythmias.

For every heartbeat, a complex interplay is required. The fundamental components are the electrical currents that control the heart's contractions and pump blood throughout the body.

Delving deeper into how these electrical currents cause the heart to pump, we encounter the cleverly designed ion channels. Ion channels are small pathways in the heart that facilitate and regulate the electrical currents controlling the heart's contractions.

However, if we dive even deeper into the heart, our understanding becomes limited. Surrounding the ion channels are other proteins that are crucial for the function of these channels.

But until now, we haven't known much about them. With a new study of mouse hearts, we are gaining more knowledge. Researchers have mapped the protein networks of the 13 most important ion channels in the heart. The study's leader is Professor MSO Alicia Lundby from the Biomedical Institute. The work is published in the journal *Nature Cardiovascular Research*.

"There are more than 800 proteins in the overall network. We have created a map of the proteins that are part of the ion channel network," says Alicia Lundby. "I was truly surprised by the number of proteins in the networks of the ion channels we have studied. One can think of protein networks somewhat similarly to social networks. We humans participate in social networks, and the people we interact with influence our actions. Similarly, the function of ion channels is influenced by the proteins they are networked with."

This mapping is fundamental for understanding how the heart's function can be regulated, leading to the development of precise heart medication for people whose hearts don't beat as they should, known as cardiac arrhythmias. These disorders can be challenging to live with and may even pose a risk of cardiac arrest.

"We want to understand which proteins are important for the function of ion channels. We believe that such insight will provide crucial knowledge for identifying new drug targets in the treatment of cardiac arrhythmias. A first step in that direction is to identify the proteins that form networks with ion channels and then prioritize these proteins based on their functional impact," says Lundby.

Molecular fishing rod

Alicia Lundby and her colleagues have worked with heart tissue from mice. They used antibodies to extract ion channels and their networks

from the heart tissue for examination.

"Antibodies are molecules that can recognize and bind proteins with high specificity. We used antibodies that specifically bind to the ion channels found in the heart. You can think of the antibody as a sort of molecular fishing rod used to catch the ion channels and their protein networks from the tissue," explains Lundby.

After extracting the ion channel networks from the heart tissue, the researchers used mass spectrometry to analyze which proteins were present in the samples.

"Mapping the ion channel networks was a significant effort. However, understanding the functional significance of these proteins is an even greater task. To prioritize among the network's proteins, we used information from large genomic studies that identify regions in the genome important for heart rhythm," says Lundby.

By combining genetic information with information about the proteins in the ion channel networks, the researchers could prioritize the proteins that appeared to have the greatest impact on ion channels.

Discovering new proteins that affect the heart's ion currents is not an everyday occurrence

The researchers investigated 10 of these proteins in animal models and then confirmed that precisely those ten proteins are important for the heart's electrical currents. They also discovered that two of the proteins they examined specifically affect the ion channel in the heart responsible for sodium flow.

"The sodium current is crucial for our heart's ability to beat, and finding

two unknown interaction partners for it is actually quite significant," says Lundby, continuing "As basic researchers, it's a big deal for us to discover new proteins that influence ion channel currents. Moreover, the sodium channel is a very important pharmacological target used in the treatment of heart patients."

The primary currents in the heart are sodium, potassium, and calcium currents. There are many different ion channels, but the heart has one primary ion channel responsible for directing the sodium current.

Lundby emphasizes that more research is needed to get closer to developing new heart medication for patients with cardiac arrhythmias.

"We need a deeper mechanistic understanding of how these two proteins affect the channel's function. We have found them, and we have shown that they directly influence the channel's function, but we still don't know exactly how they do it."

Alicia Lundby and her colleagues have already begun new experiments involving these two proteins.

More information: Svetlana Maurya et al, Outlining cardiac ion channel protein interactors and their signature in the human electrocardiogram, *Nature Cardiovascular Research* (2023). DOI: 10.1038/s44161-023-00294-y

Provided by University of Copenhagen