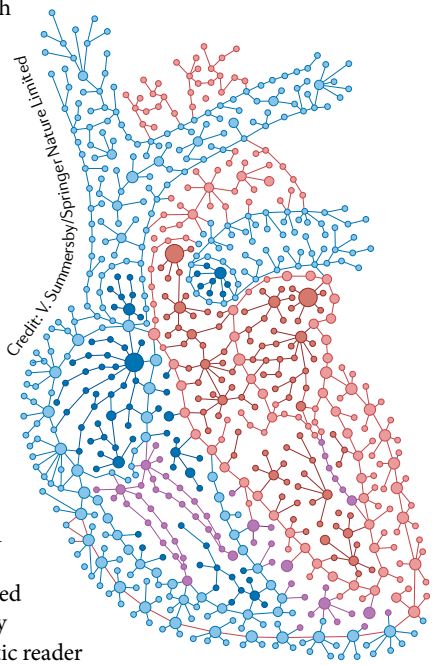
GENETICS

Protein interactomes uncover new genetic causes of CHD

A novel strategy to analyse the protein interactomes of transcription factors whose variants cause congenital heart disease (CHD) identifies *GLYR1* as a key gene in heart development, according to findings published in *Cell*. This integrative approach, which combines techniques from genetics, computational biology and proteomics, provides a framework for identifying specific combinations of genetic variants that cause complex diseases such as CHD.

The aetiology of CHD is complex owing to extensive genetic heterogeneity; >390 genes are thought to be involved in the pathogenesis of the disease. Heart defects have been linked to variants in genes encoding cardiac transcription factors. "My laboratory previously found that the transcription factor GATA4 was mutated in a large family with CHD occurring across five generations," explains Deepak Srivastava, the lead author of the study. "We also found that GATA4 interacts physically with a second transcription factor, TBX5, and later, using induced pluripotent stem cells from family members, we showed that GATA4 and TBX5 broadly bind to common regulatory elements across the genome, regulating thousands of genes and providing a potential mechanism for the morphogenic defect," he adds.

On the basis of these findings, the authors sought to map out the network of interactions between the GATA4 and TBX5 proteins (known as protein interactomes) with the use of human induced pluripotent stem cell-derived cardiac progenitors. The resulting 273-protein network was integrated with data from nearly 9,000 exomes from proband–parent trios, revealing an enrichment within the GATA4 and TBX5 protein interactome of de novo missense variants linked with CHD. The investigators subsequently designed an integrative prioritization scoring system to rank the CHD-associated variants. This strategy identified six variants in proteins with yet undescribed roles in cardiac development (two variants of BRD4, SMARCC1, GLYR1, CSNK2A1 and SAP18). The investigators focused on the ubiquitously expressed epigenetic reader



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CLINICAL TRIALS

An all-virtual clinical trial to assess a heart failure drug

The CHIEF-HF trial, a randomized placebo-controlled study that was conducted remotely without in-person interactions between doctors and patients, successfully demonstrated that the sodium–glucose cotransporter 2 (SGLT2) inhibitor canagliflozin significantly reduces symptom burden in patients with heart failure (HF). This novel trial design could improve the way in which clinical studies are conducted in the future, by reducing costs and by increasing the speed of data acquisition.

"At the time this trial was designed, there was limited information on the health status benefits of the SGLT2 inhibitors in patients with HF," explains John Spertus, first author of the study. "We thus worked with Janssen to design a clinical trial without any face-to-face visits to simplify the process of participating in a clinical trial for patients," he adds.

Given the recent qualification of the Kansas City Cardiomyopathy Questionnaire (KCCQ) by the FDA as a clinical outcome assessment and the fact that KCCQ data can be collected using smartphones, the CHIEF-HF trial investigators sought to conduct a completely decentralized virtual study to evaluate whether canagliflozin improves symptom burden in patients with HF. In total, 476 participants with HF were randomly assigned to canagliflozin (100 mg daily) or placebo. The participants provided informed consent via a mobile phone app, and the study drug and wearable activity monitor were shipped directly to their homes. Patients were asked to report the number of days that they took the study drug via the app, and they completed the KCCQ over the 12-week treatment period. The patients' symptom reports were assessed at weeks 2, 4, 6 and 12 after initiation of the drug.

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At 12 weeks, the improvement in KCCQ total symptom score was significantly greater in canagliflozin-treated patients than in placebo-treated patients. The reduction in symptom burden with canagliflozin was consistent across the range of ejection fraction and in patients with or without diabetes mellitus. Of note, the difference in KCCQ total symptom score between

GLYR1, whose role in the heart was previously unexplored. GLYR1 and GATA4 were found to co-bind and co-regulate a defined set of genes associated with heart development, and the P495L missense variant in *GLYR1* disrupted this interaction with GATA4. This finding was further confirmed in mouse studies, whereby *Glyr1*$^{P495L/+}$ mice crossed with *Gata4*$^{+/-}$ mice had cardiac septal defects.

"The authors describe a novel algorithm for gene prioritization of genomic sequencing data by integrating information from protein–protein interactome networks from known CHD-causing genes," comments Vidu Garg (Nationwide Children's Hospital, USA), who was not involved in the study. "These types of bioinformatic approaches will be an important component for the successful, widespread application of genomic sequencing information into the clinical setting".

Karina Huynh

ORIGINAL ARTICLE Gonzalez-Teran, B. et al. Transcription factor protein interactomes reveal genetic determinants in heart disease. *Cell* https://doi.org/10.1016/j.cell.2022.01.021 (2022)

the treatment groups was apparent as early as 2 weeks after starting drug therapy.

Importantly, this trial was initiated 2 weeks before a US national shutdown related to the COVID-19 pandemic. "The decentralized and completely virtual nature of this trial exemplifies how novel strategies for trial implementation might be helpful in circumstances in which traditional trials are difficult to conduct, such as during the COVID-19 pandemic," comments Prakriti Gaba (Brigham and Women's Hospital, USA), who was not involved in the study. "Moreover, the investigators noted greater generalizability by enrolling a higher number of women and minorities, a longstanding limitation of traditional clinical trials," she adds.

Karina Huynh

ORIGINAL ARTICLE Spertus, J. A. et al. The SGLT2 inhibitor canagliflozin in heart failure: the CHIEF-HF remote, patient-centered randomized trial. *Nat. Med.* https://doi.org/10.1038/s41591-022-01703-8 (2022)
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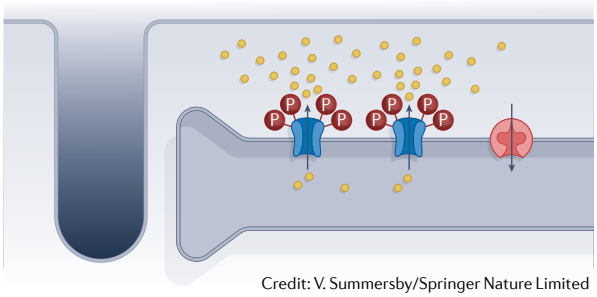
ATRIAL FIBRILLATION

Atrial fibrillation induces functional remodelling of the left ventricle



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Clinical data indicate that atrial fibrillation (AF) can cause left ventricular (LV) dysfunction and heart failure (HF). However, whereas the pathogenesis of AF has been broadly studied in the atria, the effects of AF on LV function are poorly understood. A new study now demonstrates that AF alone, in the absence of tachycardia, causes functional remodelling of the left ventricle via impairment of excitation–contraction coupling of ventricular cardiomyocytes, which is mediated by reduced systolic Ca^{2+} release and increased levels of oxidative stress. "We unravel the chicken-and-egg principle of AF and HF, showing that the arrhythmic ventricular excitation caused by AF leads to a structural and functional disturbance of ventricular function," highlight lead investigators Steffen Pabel and Samuel Sossalla.

To assess the effects of AF on LV function, the investigators analyzed samples of LV myocardium from patients with aortic stenosis with preserved LV function and with either sinus rhythm or rate-controlled AF. The amount and distribution of fibrosis in LV samples from patients with sinus rhythm was similar to the amount and distribution in samples from patients with AF. However, LV cardiomyocytes from patients with AF had altered Ca^{2+} homeostasis, with reduced systolic Ca^{2+} release (but no changes in diastolic Ca^{2+} levels and Ca^{2+} transient kinetics) and prolonged action potential duration. These findings were confirmed in isolated LV cardiomyocytes from donors with non-failing hearts with sinus rhythm or AF.

Given that the clinical characteristics of the patients might have influenced the observed phenotypes, the researchers performed in vitro experiments of AF stimulation (using arrhythmic or rhythmic pacing at 60 bpm) in human isolated primary cardiomyocytes and human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). These in vitro models confirmed the detrimental phenotypes associated with AF observed in clinical samples. After 24 h of AF simulation, human LV cardiomyocytes from non-failing hearts showed impaired Ca^{2+} transient amplitude. In iPSC-CMs, 7 days of AF simulation led to a depression in systolic Ca^{2+} transient amplitude, as well as decreased Ca^{2+} load and increased diastolic Ca^{2+} leak from the sarcoplasmic reticulum. AF simulation also induced increases in cytosolic Na^{+} concentration and the late Na^{+} current, which led to prolongation of the action potential duration.

Mechanistic studies indicated that reactive oxygen species (ROS)-dependent activation of Ca^{2+}/calmodulin-dependent protein kinase IIδc (CaMKIIδc) might contribute to the adverse remodelling of the left ventricle in AF. LV myocardium from patients with AF had higher ROS levels than LV myocardium from patients with sinus rhythm. Furthermore, the LV myocardium from patients with AF had increased oxidation of CaMKII, which led to higher CaMKIIδc activity and hyperphosphorylation of the ryanodine receptor 2. CaMKIIδc inhibition and ROS scavenging in iPSC-CMs prevented the impairment in systolic Ca^{2+} handling after AF simulation.

"This translational study provides the first mechanistic characterization of the effects of AF on the human ventricle," comment Pabel and Sossalla. The study also provides a potential mechanistic explanation for the clinical trial findings showing that rhythm control therapy improves contractility in patients with HF with reduced ejection fraction and concomitant AF. The investigators plan to conduct a large clinical trial (the TACHY-HF trial) to better understand AF-induced HF. "This trial aims to facilitate better diagnosis to characterize patients with definitive arrhythmia-induced cardiomyopathy. An early diagnosis will enable better tailoring of therapy and thereby avoid unnecessary treatments and complications in non-responders," explains Sossalla. "The results of this trial will also shed light on the general characteristics of the disease, the clinical course, the complications and the risk of recurrence, as a basis for future interventional studies," he concludes.

Irene Fernández-Ruiz

ORIGINAL ARTICLE Pabel, S. et al. Effects of atrial fibrillation on the human ventricle. *Circ. Res.* https://doi.org/10.1161/CIRCRESAHA.121.319718 (2022)
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