Case study

HT Screening to Classify and Stratify Cardiac Channelopathy Variants

Tools:
SyncroPatch 384

Chai-Ann Ng, PhD
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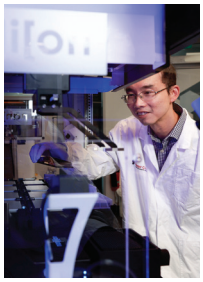


Chai-Ann Ng, PhD is a Senior Staff Scientist at the Victor Chang Cardiac Research Institute and Conjoint Senior Lecturer at UNSW. His research integrates electrophysiology, and cell biology to study hERG potassium channels. He develops high-throughput methods to assess pathogenicity of genetic variants in Long QT Syndrome and to identify small-molecule therapeutics for arrhythmia treatment.

Cardiac channelopathies such as Long QT Syndrome (LQTS) and Brugada Syndrome are inherited arrhythmia syndromes that commonly run in families. These conditions can lead to symptoms ranging from fainting spells to sudden cardiac arrest, which may be fatal if not treated immediately. Therefore, timely access to an Automated External Defibrillator (AED) and the ability to perform cardiopulmonary resuscitation (CPR) are vital emergency measures. Therapeutic strategies, including the use of β-blockers and Implantable Cardioverter-Defibrillators (ICDs), have been shown to significantly reduce mortality in symptomatic individuals. Genetic testing plays an important role in confirming diagnoses and identifying at-risk, asymptomatic family members. However, it also introduces new challenges, particularly when variants of uncertain significance (VUS) are identified - variants whose clinical importance remains ambiguous.

At the Victor Chang Cardiac Research Institute in Sydney, Australia, the Cardiac Electrophysiology Laboratory, led by Professor Jamie Vandenberg and supported by Senior Staff Scientist Dr. Chai-Ann Ng, focuses on the functional assessment of cardiac ion channel variants. Their research offers vital support to clinical genetic testing laboratories in Australia, the United States, Europe and the United Kingdom, helping to improve the accuracy of genetic diagnoses in patients with cardiac channelopathies. In parallel, the team collaborates with clinicians and researchers to enhance risk stratification for LQTS and refine penetrance estimates for Brugada Syndrome.

Each genetic variant identified in a patient represents a unique piece of a much larger puzzle. Only through large-scale functional assessment of these variants can researchers gain a comprehensive understanding of disease complexity and the diverse risk levels observed across patients. To date, the team has screened over 1,000 cardiac ion channel variants, underscoring the scale and depth of their investigations.

Patch clamp electrophysiology is the gold standard for determining the function of ion channel variants. However, manual patch clamp techniques are limited because of their

Nanion's SyncroPatch 384 platform
(successor of SyncroPatch 384PE and SyncroPatch 384i) allows effortless high-throughput ion channel screening coupled with high flexibility and reliability, due to the combination of its patch clamp module with Beckman Coulter's new i-Series liquid handling robot Biomek i5.

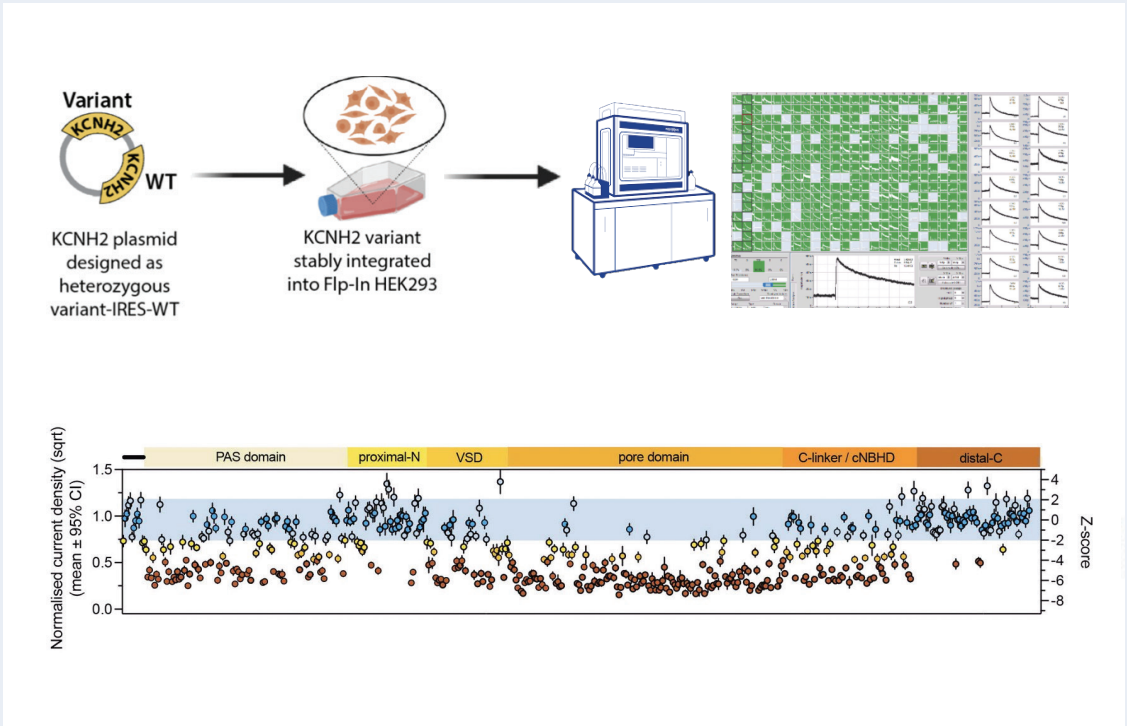
> **"Screening over a thousand ion channel variants, with SyncroPatch 384, has transformed our understanding of cardiac risk and is bringing real answers to patients previously left in diagnostic limbo."**
>
> **Prof. Jamie Vanderberg,** Victor Chang Cardiac Research Institute

low throughput, making it unsuitable for studying hundreds or thousands of variants. Recognizing this bottleneck, the Victor Chang Cardiac Research Institute acquired the high-throughput automated patch clamp (APC) system, the SyncroPatch 384 in 2018, which was upgraded in 2025. The acquisition was made possible through generous support from the McCusker Charitable Foundation and Commercial Projects Pty Ltd. The SyncroPatch 384 is transforming the way ion channel-related cardiac diseases are studied, enabling greater efficiency, reproducibility, and clinical relevance. This cutting-edge technology has been essential in allowing timely, large-scale functional testing.

Approximately 90% of LQTS cases are linked to mutations in three key ion channel genes: *KCNQ1*, *KCNH2*, and *SCN5A*, which correspond to LQT1, LQT2, and LQT3, respectively[1].

Although LQT3 is considered the most dangerous subtype, it accounts for only 10% of LQTS cases. LQT1 and LQT2 are more common and exhibit distinct clinical triggers. LQT1 patients are often advised to avoid strenuous physical activity or competitive sports, while LQT2 triggers are more unpredictable and include sudden auditory stimuli, emotional stress[2], and the postpartum period in women[3].

LQT2 is characterized as a loss-of-function disease resulting from reduced I_{Kr} current, leading to QT interval prolongation observable on the surface ECG. The I_{Kr} current is mediated by $K_v11.1$ potassium channels encoded by the *KCNH2* gene. In 2020, the team at the Victor Chang Institute developed the first APC-based assay capable of distinguishing between benign and pathogenic *KCNH2* variants[4]. This assay was later validated to meet current clinical standards in 2024[5]. To



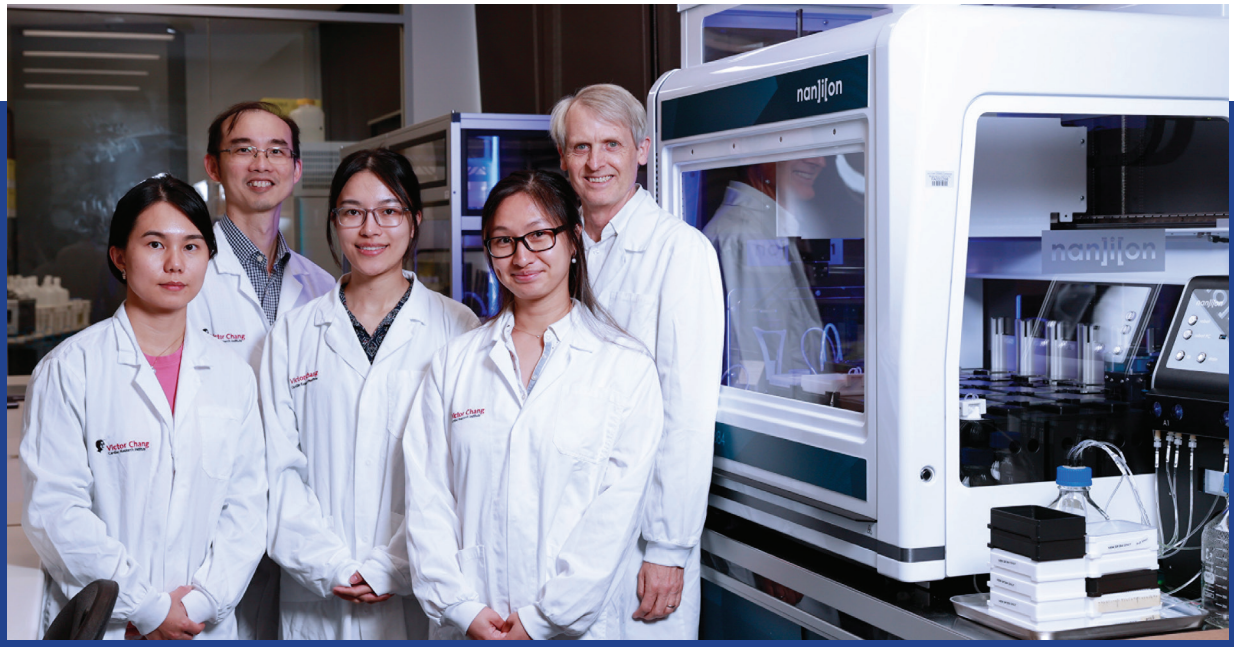
APC assessment of KCNH2 variants from LQTS patients. *All KCNH2 variants are co-expressed with WT in Flp-in HEK293, and the current amplitudes of 533 KCNH2 variants located throughout the $K_v11.1$ K^+ channel were measured using the Nanion SyncroPatch 384.*

date, over 500 *KCNH2* variants identified in patients with LQT2 through international collaborations have been analyzed using this assay. This represents the largest functional study of its kind using patch clamp electrophysiology and was published in Circulation in 2024[6].

Because LQT2 involves loss-of-function, the research team measured the current density expressed in stably transfected Flp-In HEK293 cells7[7]. These quantitative current measurements, obtained through the APC assay, were shown to be useful for risk stratification in LQT2 patients[6]. Moreover, these KCNH2 functional data were also used by clinical genetic testing laboratories in Australia, the UK[8], and the USA[9] to reclassify abnormal *KCNH2* variants as likely pathogenic or pathogenic. A notable collaboration with one of the world's largest diagnostic laboratories led to improved diagnoses for many patients with *KCNH2* variants[9]. This example illustrates how functional data can serve as robust clinical evidence, bridging the gap between laboratory research and real-world patient care.

Building on the success in LQT2, the team also developed an APC assay for Brugada Syndrome, a condition linked to loss-of-function mutations in *SCN5A*, which encodes the $Na_v1.5$ sodium channel. This was the first instance in which an APC assay was independently replicated at two institutions: the Victor Chang Cardiac Research Institute in Australia and Vanderbilt University Medical Center in the USA[10]. The assay was applied to 252 *SCN5A* variants identified through an international Brugada Syndrome cohort[11]. The resulting data demonstrated penetrance assesment of Brugada Syndrome by using only the current density of $Na_v1.5$ measured by the APC and provided validated functional data for variant reclassification in *SCN5A*[11].

These large-scale functional studies were made possible through substantial support from the Australian Government's Medical Research Future Fund and the Victor Chang Cardiac Research Institute Innovation Centre, funded by the New South Wales State Government. Central to the success of these projects was the Nanion SyncroPatch 384 APC system, which enables simultaneous assessment of up to 10 ion channel variants in a 384-well format. This high-throughput approach allows for multiple replicates across a large number of variants, generating statistically significant data suitable for clinical application.



Ion Channel Functional Genomics Team *(from the left): Dr Chek-Ying Tan, Dr Chai-Ann Ng, Miss Evie Shen, Dr Joanne Ma and Prof Jamie Vandenberg.*

"The SyncroPatch 384 is a game-changer. Its high throughput and precision have been crucial in enabling us to generate the world's largest patch clamp dataset that directly informs clinical decision-making in cardiac genetics."

Dr. Chai-Ann Ng., Victor Chang Cardiac Research Institute

"Through our pioneering efforts, we have demonstrated that high-throughput functional testing can be clinically meaningful.", says Dr. Ng. The laboratory has enabled the reclassification of hundreds of *KCNH2* and *SCN5A* variants that were previously designated as Variants of Uncertain Significance (VUS) due to lack of functional evidence. Their functional data are now accepted in clinical settings across Australia, the UK, and the USA, thanks to assays developed in accordance with the ClinGen Sequence Variant Interpretation Working Group guidelines[12], which considers biological and statistical significance.

Dr. Ng continues, "The next phase of our research aims is to expand into additional channelopathies, including *KCNQ1* and *CACNA1C*, as well as neurological channelopathies. Central to this goal is the ongoing collaboration with clinical genetic testing laboratories, ensuring that the lab's discoveries continue to reach patients quickly and effectively, bringing precision medicine closer to those who need it most."

Contact Information

Chai Ann Ng (Andy), PhD

Senior Staff Scientist, Victor Chang Cardiac Research Institute; Conjoint Senior Lecturer, School of Clinical Medicine, UNSW Medicine & Health, UNSW Sydney
405 Liverpool St, Darlinghurst NSW 2010 Australia
c.ng@victorchang.edu.au
https://www.victorchang.edu.au/chaiann-ng

Prof Jamie Vandenberg, MBBS, PhD, FAHMS

Co-Deputy Director of Victor Chang Cardiac Research Institute; Head, Mark Cowley Lidwill Research Program in Cardiac Electrophysiology; Conjoint Professor, School of Clinical Medicine, UNSW Medicine & Health, UNSW Sydney
j.vandenberg@victorchang.edu.au
https://www.victorchang.edu.au/jamie-vandenberg

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