Investigation of the functional impact of *HCN2*-variants, involved in genetic Neurodevelopmental disorders

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I. Scientific context and scientific issues to be tackled

The hyperpolarization-activated cyclic nucleotide-gated (HCN) channels constitute a subfamily of voltage-gated ion channels (VGIC), with 4 members (HCN1-4), encoded by 4 genes (HCN1-4) which are mainly expressed in the brain and the heart[1]. HCN channels are activated by hyperpolarized membrane potential and conduct very slow depolarizing currents mediated by Na^+ and K^+, called I_f in cardiomyocytes and I_h in neurons and regulated by cAMP[1]. Of note, HCN2 channels is the most abundantly expressed and broadly distributed throughout the brain, remarkably in the cortex, in the hippocampus, the amygdala, and in the thalamus, brain regions, underlying the pathophysiology of neuronal disorders, such as epilepsy and neurodevelopmental disorders (NDDs)[2,3]. HCN2 subunits form homotetramer, but they are also present in association with HCN1 in cortical structures[4–6]. Of note, the increased formation of HCN1/HCN2 heteromeric channels in cortical neurons is associated with altered properties of the resulting Ih, significantly enhancing network excitability[4–6]. In addition, HCN1 and HCN2 homotetramers exhibits distinct subcellular localization in pyramidal neurons, while HCN1 is mostly dendritic, HCN2 is expressed in the soma[7]. HCN1/HCN2 heterotetramers are localized in dendrites in these neurons.

Although the number of *HCN1* and *HCN2* variants recorded in genetic databases such as ClinVar is growing, the pathophysiology of most of these variants is still unknown. Today, the highest number of pathogenic variants linked to NDDs and epilepsy is found for HCN1[8,9]. More than 30 pathogenic variations in HCN1 have been clearly established as a cause of epilepsy, but also of intellectual deficiency (ID)[8]. Indeed, *HCN1* variants have been described in *in-vitro* studies and using transgenic models, showing loss of function (LoF), gain of function (GoF) and aberrant function (AF)[7,10,11]. All insights concerning the functional impact of *HCN1* and *HCN2* variants were obtained independently. **Although HCN1/HCN2 heterotetramers are present in cortical and pyramidal neurons, the functional impact of HCN2 variants on HCN1/HCN2 heterotetramers and vice versa remains unknown.**

In contrast, before our last report[12], only 6 *HCN2* pathogenic variants (4 GoF and 2 LoF) have been described in a restricted number of individuals, with mild epilepsies, mostly without ID [13–17]. In our recent study, we collected the clinical features of 21 individuals from 15 unrelated families, carrying 13 *HCN2* variants, with NDDs with and without epilepsy[12]. Using *Xenopus* oocytes as heterologous expression system, we characterized 6 variants: 1 GoF variant (R234H), 3 LoF (L377H, P493L, G587D) and 2 AF variants (A363V, M374L) in *HCN2*[12] by electrophysiology. In addition, we studied the structural impact of these variants on the 3D-structure of HCN2 channels. More recently, we enlarged our cohort with 5 novel patients (unpublished data), carrying *HCN2* variants, with NDDs with and without epilepsy. The aim of our group is to further investigate the functional and structural impacts of *HCN2* variants on HCN1 and HCN2 function, by combining different *in-vitro* and *in-silico* approaches.

II. Missions of the student

Thus, our cohort reaches 26 patients today, and 19 *HCN2* novel variants were identified. The student will contribute to :

1) Characterizing the functional impact of 19 *HCN2* variants in *in-vitro* assays and gathering the clinical features of 26 patients with NDDs with or without epilepsy

2) Investigating the impact of *HCN2* variants on HCN1 function by co-expression experiments in heterologous systems (HEK293T, SH-SY5Y cells)

3) Investigating the impact of *HCN2* variants on HCN2 and HCN1 channels 3D-structure

The study will be conduct by combining electrophysiology (patch-clamp recordings), 3D modelling and molecular cell biology (cell culture, confocal microscopy, western blot, RT-qPCR…).

III. Conditions of the internship (location, potential compensation or advantages)

The internship will take place at our research laboratory, called Mitovasc, located within the Hospital University center of Angers, a multidisciplinary center equipped with state-of-the-art facilities for molecular biology, electrophysiology, and imaging. The student will be integrated into a dynamic and collaborative research team (https://mitovasc.univ-angers.fr/en/index.html) working on ion channel physiology and neurodevelopmental disorders. The intern will benefit from: 1) Full access to laboratory equipment and resources; 2) Supervision and training by experienced researchers in electrophysiology, molecular biology, and structural modeling; 3) Participation in weekly lab meetings and seminars; 4) Support for accommodation and transportation may be available depending on the funding scheme and institutional agreements; 5) A certificate of completion and potential co-authorship on publications, depending on the contribution. In addition, the working language of the lab is English, and the environment is international and inclusive.

IV. Link with the Sustainable Development Goal(s) and the global EU GREEN vision

This internship aligns with several Sustainable Development Goals (SDGs), such as:

- **SDG 3: Good Health and Well-being:** By investigating the molecular mechanisms underlying NDDs and epilepsy, this project contributes to the advancement of biomedical knowledge that can inform future therapeutic strategies and improve patient outcomes.
- **SDG 4: Quality Education:** The internship provides high-level scientific training and promotes international academic mobility, fostering the development of skilled researchers in the field of neuroscience.
- **SDG 9: Industry, Innovation and Infrastructure:** The project integrates innovative methodologies such as 3D structural modeling and advanced electrophysiological techniques (TEVC and patch-clamp electrophysiology), contributing to the development of cutting-edge research infrastructure.

In addition, in line with the EU GREEN vision, this internship will promote to: 1) Interdisciplinary collaboration across molecular biology, biophysics, and clinical genetics; 2) Sustainable academic partnerships by strengthening ties between European institutions and 3) Open science and data sharing, as the project contributes to publication in open access journal, publicly available genetic and clinical databases.

V. References (in bold publication of the team)

1. Mishra P, Narayanan R. The enigmatic HCN channels: A cellular neurophysiology perspective. *Proteins*. 2023;93(1):72-92. doi:10.1002/prot.26643
2. Parenti I, Rabaneda LG, Schoen H, Novarino G. Neurodevelopmental Disorders: From Genetics to Functional Pathways. *Trends in Neurosciences*. 2020;43(8):608-621. doi:10.1016/j.tins.2020.05.004
3. Kessi M, Peng J, Duan H, et al. The Contribution of HCN Channelopathies in Different Epileptic Syndromes, Mechanisms, Modulators, and Potential Treatment Targets: A Systematic Review. *Front Mol Neurosci*. 2022;15:807202. doi:10.3389/fnmol.2022.807202
4. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med*. 2001;7(3):331-337. doi:10.1038/85480
5. Chen S, Wang J, Siegelbaum SA. Properties of Hyperpolarization-Activated Pacemaker Current Defined by Coassembly of Hcn1 and Hcn2 Subunits and Basal Modulation by Cyclic Nucleotide. *The Journal of General Physiology*. 2001;117(5):491-504. doi:10.1085/jgp.117.5.491
6. Brewster AL, Bernard JA, Gall CM, Baram TZ. Formation of heteromeric hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in the hippocampus is regulated by developmental seizures. *Neurobiology of Disease*. 2005;19(1-2):200-207. doi:10.1016/j.nbd.2004.12.015
7. Santoro B, Shah MM. Hyperpolarization-Activated Cyclic Nucleotide-Gated Channels as Drug Targets for Neurological Disorders. *Annu Rev Pharmacol Toxicol*. 2020;60(1):109-131. doi:10.1146/annurev-pharmtox-010919-023356
8. Bleakley LE, Reid CA. *HCN1* epilepsy: From genetics and mechanisms to precision therapies. *Journal of Neurochemistry*. 2024;168(12):3891-3910. doi:10.1111/jnc.15928
9. Han Z, Xie L, Liu X, et al. Characterization of the phenotype and functional alternations of three HCN1 variants in Chinese epilepsy patients. *Epilepsy Research*. 2025;211:107521. doi:10.1016/j.eplepsyres.2025.107521
10. Bleakley LE, McKenzie CE, Soh MS, et al. Cation leak underlies neuronal excitability in an HCN1 developmental and epileptic encephalopathy. *Brain*. 2021;144(7):2060-2073. doi:10.1093/brain/awab145
11. Bleakley LE, McKenzie CE, Aung KP, et al. Org 34167 rescues voltage dependence of mutant channels and normalizes hyperexcitability in *HCN1* epilepsy. *Epilepsia*. Published online August 5, 2025:epi.18585. doi:10.1111/epi.18585
12. **Houdayer C, Phillips AM, Chabbert M, et al. *HCN2*-Associated Neurodevelopmental Disorders: Data from Patients and *Xenopus* Cell Models. *Annals of Neurology*. 2025;98(3):573-589. doi:10.1002/ana.27277**
13. Dibbens LM, Reid CA, Hodgson B, et al. Augmented currents of an HCN2 variant in patients with febrile seizure syndromes. *Ann Neurol*. 2010;67(4):542-546. doi:10.1002/ana.21909
14. DiFrancesco JC, Ragona F, Murano C, et al. A novel de novo *HCN2* loss-of-function variant causing developmental and epileptic encephalopathy treated with a ketogenic diet. *Epilepsia*. 2023;64(12). doi:10.1111/epi.17777
15. DiFrancesco JC, Barbuti A, Milanesi R, et al. Recessive Loss-of-Function Mutation in the Pacemaker HCN2 Channel Causing Increased Neuronal Excitability in a Patient with Idiopathic Generalized Epilepsy. *J Neurosci*. 2011;31(48):17327-17337. doi:10.1523/JNEUROSCI.3727-11.2011
16. Li M, Maljevic S, Phillips AM, et al. Gain-of-function *HCN2* variants in genetic epilepsy. *Human Mutation*. 2018;39(2):202-209. doi:10.1002/humu.23357
17. Nakamura Y, Shi X, Numata T, et al. Novel HCN2 Mutation Contributes to Febrile Seizures by Shifting the Channel's Kinetics in a Temperature-Dependent Manner. Attali B, ed. *PLoS ONE*. 2013;8(12):e80376. doi:10.1371/journal.pone.0080376