

# Patient-derived neuronal models for pharmacogenetic pain treatment of sodium channelopathies

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# CHAPTER 7

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## SIGNIFICANCE STATEMENT



## CLINICAL AND SCIENTIFIC IMPACT

Chronic pain affects nearly 1 in 4 individuals worldwide [1-3], and despite its global spread, treatments for this condition are still largely ineffective and carry serious side effects [4, 5]. Recent drug developments have focused on inhibitors of voltage-gated sodium channels (VGSC), which, when dysfunctional, are associated with several human pain syndromes [6-8]. Different classes of VGSC blockers have emerged, including anticonvulsants, local anesthetics, and isoform-selective blockers. Lacosamide, an anticonvulsant and emerging candidate for treating pain [9], has shown variable efficacy in patients with Nav1.7-related small fiber neuropathy (SFN) [10]. To date, the peripheral mechanisms underlying lacosamide treatment response modulation are still poorly understood. With limited number of individuals responsive to available medications, unraveling the contributing factors interfering with pain targets and drug modulation is highly warranted. In **chapter 2**, we recapitulated inter-individual variations in lacosamide response in patients with Nav1.7 mutations. Our results support the utilization of a pre-clinical biophysical assessment of specific mutations to select compounds to predict individualized patient response. Furthermore, our -and similar- findings can be extrapolated back to the clinic for patients genotyped with already-assessed Nav1.7 mutations, allowing clinicians to anticipate their response to, in this case, lacosamide. We also linked specific genetic mutations to distinct pharmacological phenotypes in different SFN patients, which can be incorporated in future studies to develop better-suited treatment strategies. Epilepsy research has taught us important lessons on the extrapolation of genomic data for pharmacotherapy, by titrating dosages based on Nav1.7 splicing variants [11]. A comparable, easy-to-use pharmacogenomic-based model is proposed here for pain, which can as well be implemented to study a wide range of mutations and drugs, whereby the biophysical signature correlating to a positive drug response can guide treatment.

Understanding the mechanisms of action of analgesics, such as lacosamide and its isoform-selectivity, is critical to make informed decisions when treating specific patients and could aid future drug developments. Lacosamide has been shown to exert its effects in an unconventional manner compared to traditional pore-blocker sodium channel inhibitors [12-16]. Solving its mechanistic basis can further explain inter-individual variability in efficacy and tolerability in treated patients [10]. **Chapter 3** provides a description of a working model of lacosamide and reports a novel binding region in Nav1.7, in the voltage-sensing domain 4 (VSD4), which, alongside an intact local anesthetic site within the channel's pore, is required for lacosamide to exert its therapeutic functions. The role of the VSD4 seems to be determinant to lacosamide-isoform specificity at clinically-achievable concentrations. By unraveling the interaction between lacosamide and the VSD4, **chapter 3** sheds a light on possible implications in other sodium channelopathies, where other VGSC isoforms are affected, for example in patients with VGSC-related epilepsy or cardiac disease. By establishing the binding properties of lacosamide, we can make better predictions on drug efficacy and tolerability in patients. Our findings also highlight the pivotal role the

VSD play in initiating and maintaining lacosamide functions at therapeutic concentrations, which may be relevant in patients with mutations located in the VSD. Specifically, our results show that in cells expressing the W1538R variant, the mutation-related lacosamide block can be overcome by increasing dosage. Therefore, genomic evaluation of neuropathic pain patients, whereby specific genetic variants in Nav1.7 are detected, can be used as a dosing guideline.

Modeling genetic diseases is a tedious task, as there is a constant need for further developing cellular systems mimicking those in physiological conditions. The use of patient-specific cells can extend our understanding of pain pathophysiological mechanisms [17-20] and drug response [17, 21, 22]. In **chapter 4**, we initiated the development of a dorsal root ganglion (DRG)-targeted *in vivo* and *ex vivo* differentiation method, as a model of “humanized” cells, to offer a more translational approach, which could potentially help increase the success rates in clinical trials. While we did not succeed in forcing iPSC differentiation *in vivo* yet, successful optimization of our iPSC methods could inform us on iPSC patterns of migration, speed of differentiation and body absorption. Furthermore, this may allow drug screening in iPSCs, which will in turn extend our knowledge on iPSC-derived cells metabolism in response to drug exposure, and further delineate the responsiveness of alternative variants to different VGSC-blocking treatments. Furthermore, our results were educational on inter-species physiological interactions and on iPSC behaviors and parameters that affect DRG teratoma development. Our study provided substantial data on the differentiation and migration properties of undifferentiated cells in the rat DRG and its potential for tumorigenicity, as well as its influence on non-transplanted adjacent organs and overall physiological functions. Although beyond the scope of this thesis, these findings can serve as a basis for stem cell therapy in the DRG on how to avoid cell graft rejection and tumor formation, which can have major implications for advances in regenerative medicine. Furthermore, our model provides important insights to the study of iPSCs that are transplanted into a specific organ and can be used to propel research efforts in other pain-related or unrelated diseases.

Besides concerns associated with iPSC immaturity, the lack of functional expression of the Nav1.8 and Nav1.9 sodium channels in iPSC-derived sensory neurons (iPSC-SN) still represent a prominent caveat to the study of painful sodium channelopathies in iPSC models [18, 23]. These two channels are particularly important to pain signaling as they contribute to a large portion of the sodium current transmission and cellular excitability [24, 25]; therefore, without their physiological input, the recorded current is corrupted and not representative of the patient’s cells true electrophysiological properties. In **chapter 5**, we described for the first time the relationship and contribution of human Nav1.8 and Nav1.9 channels to human neuronal excitability as pain correlates. Furthermore, not only were we able to produce Nav1.8 and Nav1.9 currents, but we also developed a brand-new model allowing the study of Nav1.8 and Nav1.9 mutations in iPSC-SNs via dynamic clamp, which was never possible before. Moreover, this technique can be used to introduce several mutations simultaneously,

in the same patient-specific cells, covering a wider range of genetic variants and possible interactions. In turn, patients who carry genetic variants in the two channels can be evaluated in the future. Additionally, the overall cellular excitability of patient iPSC-SNs with Nav1.7 mutations might be restored to similar levels that would have been recorded in the donor's neurons, and thus, aid find better fitted treatments. Our ability to modulate Nav1.8 and Nav1.9 via dynamic clamp may further guide future therapeutic considerations in drug development and clinical trial dosing. Specifically, dynamic clamp of iPSC-SNs might bridge the gap in knowledge regarding the precise degree of channel inhibition needed to induce therapeutic relief without total loss of pain sensation [26, 27].

Taken together, the findings from this thesis have set the stage for using pharmacogenomic-guided tools in patient-specific iPSC-SNs to solve pain mismanagement while providing novel strategies to implement personalized therapy in pain research.

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