Scientific Center for Neuropathic pain - Aachen
Sodium Channel Network Aachen

Targeting NaV1.7: How Mechanism-Based Multidisciplinary Research Drives Precision Therapy in Inherited Pain Syndromes – A Translational Approach

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Rationale and medical need

Neuropathic pain affects the quality of life of around 8% of the population, but treatment has made only limited progress over the last two decades. Thus, an innovative approach is needed to meet this medical need. At the Scientific Center for Neuropathic Pain Aachen (SCN[AACHEN]) we have established an interdisciplinary analysis platform/pipeline to investigate individual sodium channel variants, with a focus on NaV1.7, identified in patients of our SCN[AACHEN]-patient registry suffering from small fiber neuropathy. We aim to address the treatment gap and to advance neuropathic pain research by interdisciplinary and translational research on neuropathic pain mechanisms.

Pharmacology
- High throughput patch clamp
- Multi-electrode arrays
- Repurposing

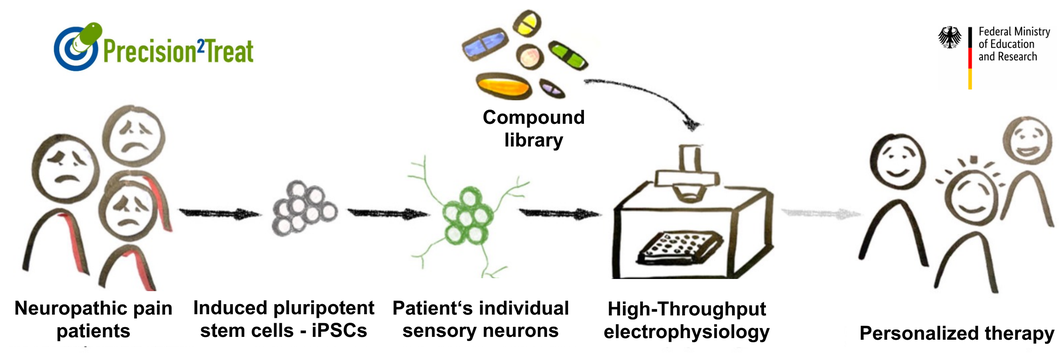
Genetic and clinical phenotyping
- Cohort of 250+ small fiber neuropathy patients
- Genetic sequencing (WES and WGS)
- In-depth Clinical and biometric data (PainWatch)
- Brain Connectivity (fMRI)

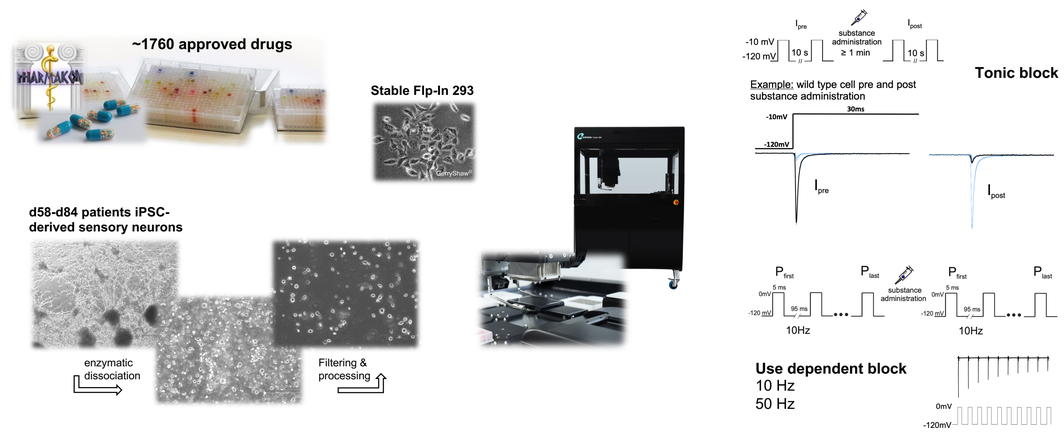
3D structural modeling
- Molecular dynamics
- Drug docking
- *In silico* mutagensis

Digital Pain Patient
- Digital human nociceptor
- Markov Models of Nav
- Predicitive models for disease progression and therapy

Nociceptor profiling
- Functional assesment with microneurography on patients
- PatchSeq of pig, human and iPSC derived sensory neurons
- Neurite RNAseq
- Nav subtype select. iPSC-nocic. classification by optogenetics

Nav variant druggabillity testing





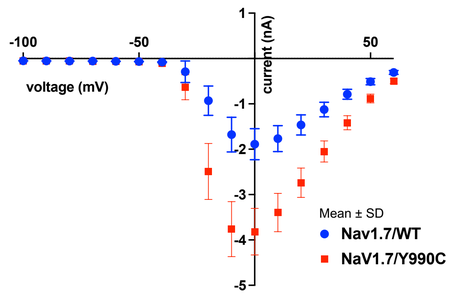
Easy-to-Clone SCN9A cDNA

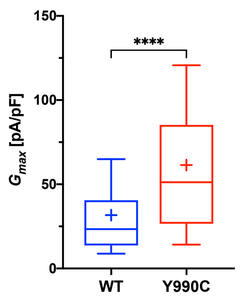
SCN9A plasmids are technically challenging as they frequently undergo rearrangements during propagation and result in very low yield after purification. Thus the efficiency of mutagenesis is usually low. To overcome this bottleneck a codon optimization strategy (Bertelli et al., 2018) was used to create an *easy-to-clone* SCN9A cDNA. SCN9A and SCN5A amino acid (aa) sequences were aligned and for each position in which the same aa was present, the corresponding codon of the SCN9A cDNA sequence was substituted by the SCN5A codon. For all other aa, the codon present in SCN9A was replaced by the synonymous codon being most similar to the corresponding SCN5A codon, if possible. The modified cDNA sequence was synthesized and subcloned into a modified pcDNA5.1/FRT/TO vector enabling Gateway®-cloning and FRT-mediated Flp-In® stable cellline generation.

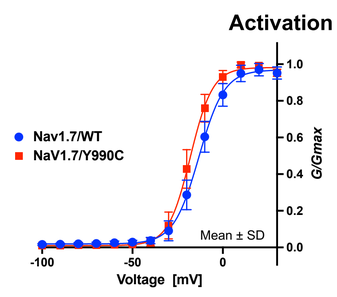
SCN5A	**^{7}P**	**R**	**G**	**T**	**S**	**S**	**F**	**R**	**R**	**F**	**T**
	^{19}cct	cgg	ggc	acc	agc	agc	ttc	cgc	agg	ttc	aca
SCN9A	**^{6}P**	**P**	**G**	**P**	**Q**	**S**	**F**	**V**	**H**	**F**	**T**
	^{16}ccc	**cca**	**gga**	**cct**	**cag**	**agc**	**ttt**	**gtc**	**cat**	**ttc**	**aca**
↓ codon optimization ↓											
SCN9A cod.opt.	**^{16}cct**	**ccg**	**ggc**	**ccc**	**cag**	**agc**	**ttc**	**gtc**	**cat**	**ttc**	**aca**
	^{6}P	**P**	**G**	**P**	**Q**	**S**	**F**	**V**	**H**	**F**	**T**

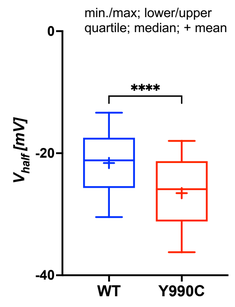
Biophysical Profiling of NaV1.7/Y990C

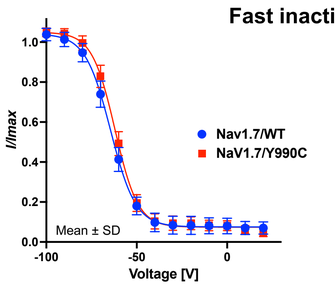
Biophysical profiling by APC of the pathogenic NaV1.7/Y990C variant associated with SFN showed a hyperpolarising shift of activation and a depolarizing shift in fast inactivation confirming a gain-of-function gating phenotype consistent with SFN. However, an initial screening of 90 approved „sodium channel" drugs showed no different effect compared to the WT channel.

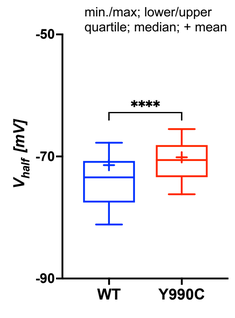






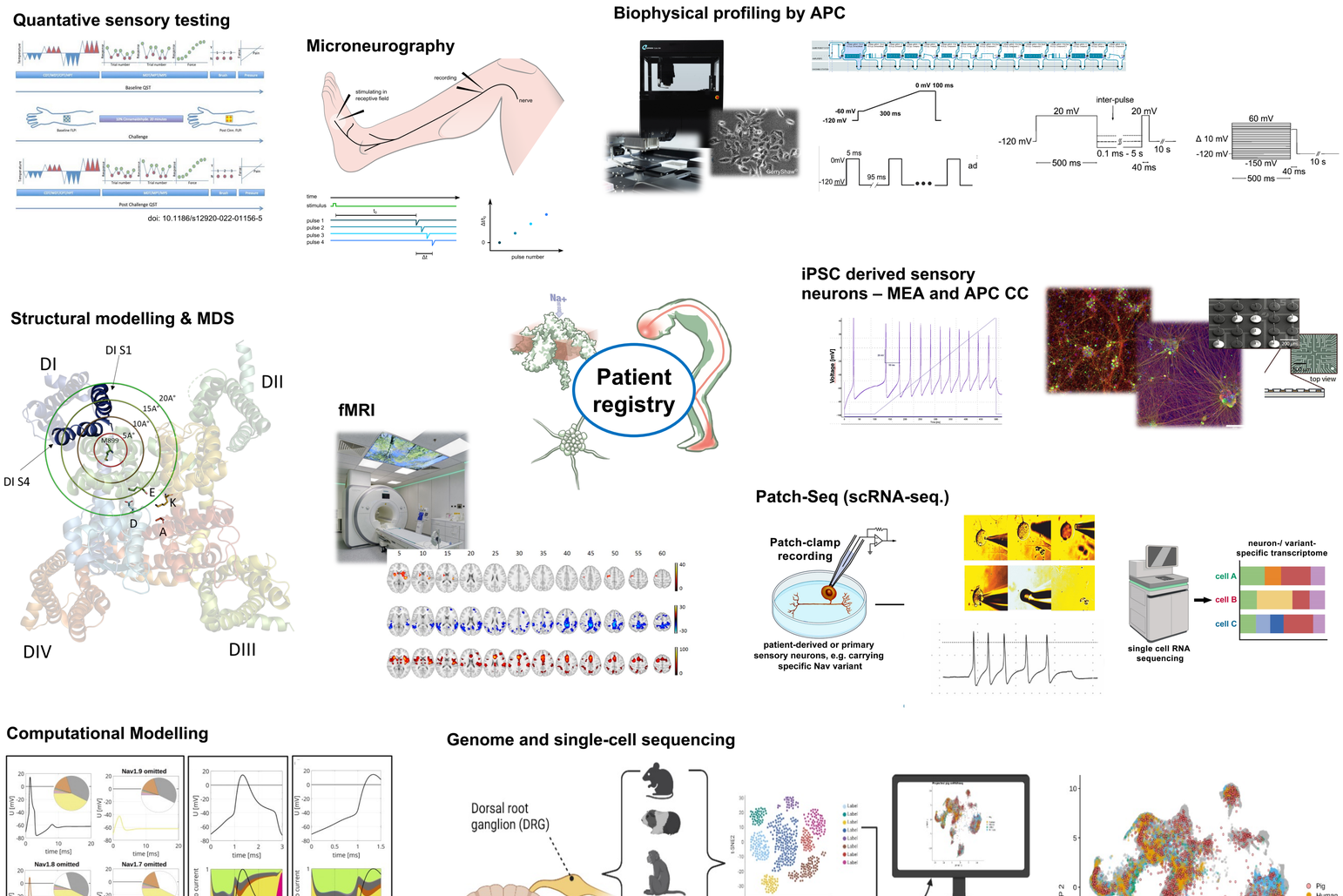






Our interdisciplinary approach

Clinical phenotyping, quantitative sensory testing, fMRI and next-generation sequencing are applied to capture patient-specific SCN9A alleles and their clinical phenotype. Variant cDNAs are inserted into a streamlined *easy-to-clone* SCN9A construct that facilitates rapid subcloning. Biophysical profiling is executed on an Qube 384 automated high-throughput patch-clamp (APC) platform in stable heterologous expression Flp-In® 293 cell lines and in iPSC-derived sensory neurons. Simultaneous molecular dynamics simulations, Hodgkin-Huxley and Markov modeling and subcellular compartmental computer modelling help to understand changes in gating and excitability, while *in vitro* high-throughput screening of already approved drugs explores whether variant-specific pharmacological treatment is possible by drug repurposing.



Summary and Outlook

The SCN[AACHEN] patient registry, NGS, high-throughput electrophysiology, drug screening, and iterative modelling provides a scalable strategy for (patho)mechanistic annotation and precision pharmacology of NaV1.7 variants. It's translation potential extends beyond the example of NaV1.7/Y990C, accelerating rational therapy development for the spectrum of NaV1.7-mediated pain disorders.

Selected References

Bertelli S et al., Gain of function of sporadic/familial hemiplegic migraine-causing SCN1A mutations: Use of an optimized cDNA. *Cephalalgia*. 2019 Apr;39(4):477-488. Köster PA et al., Nociceptor sodium channels shape subthreshold phase, upstroke, and shoulder of action potentials. *J Gen Physiol*. 2025;157:e202313526. Körner J et al., Molecular architecture of human dermal sleeping nociceptors. bioRxiv PREPRINT doi 10.1101/2024.12.20.629638.

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