WE237

Association between two *TRPM2* mutations and Painful Small Fiber Neuropathy: from NGS analysis to functional characterization

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Introduction

- Small fiber neuropathy (SFN) is a heterogeneous group of disorders affecting thin myelinated Aδ and unmyelinated C fibers
- Clinical symptoms include neuropathic pain, possible loss of thermal and nociceptive sensation, and autonomic disturbances [3]
- The aetiology of SFN is complex, involving both inherited and acquired factors
- Genetic studies in painful SFN revealed that Voltage Gated Sodium Channels (VGSC) genes, particularly SCN9A, are involved in pain amplification [1,2]
- Besides VGSC genes, Transient Receptor Potential (TRP) channel genes have also been associated with neuropathic pain because of their pivotal role in nociception [6]

Aim

To broaden genetic knowledge of painful SFN via whole-exome sequencing (WES) on Italian families with SFN and to investigate mutation impacts on channel function

Methods

- Twelve families with painful SFN were selected having at least one affected member, positive neurological examination and pain questionnaire result with numerical rating score >=4.
- After performing WES, Variants were filtered, keeping only the ones mapping to a manually curated panel of pain related genes (n=592), with minor allele frequency ≤5% in population databases [4]; conservation and computational predictors were also considered in the filtering. Segregation analysis was performed in each pedigree.
- The impact of selected causative variants was assessed through electrophysiological patch-clamp recordings on HEK cell line cultures transfected with wildtype and mutant expression vectors [5].

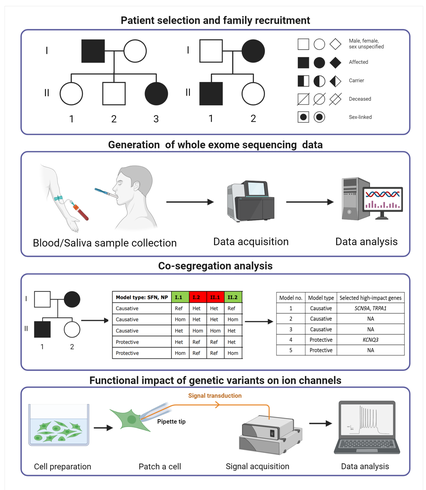

Figure 1: Graphical representation of methodology of the study

Results

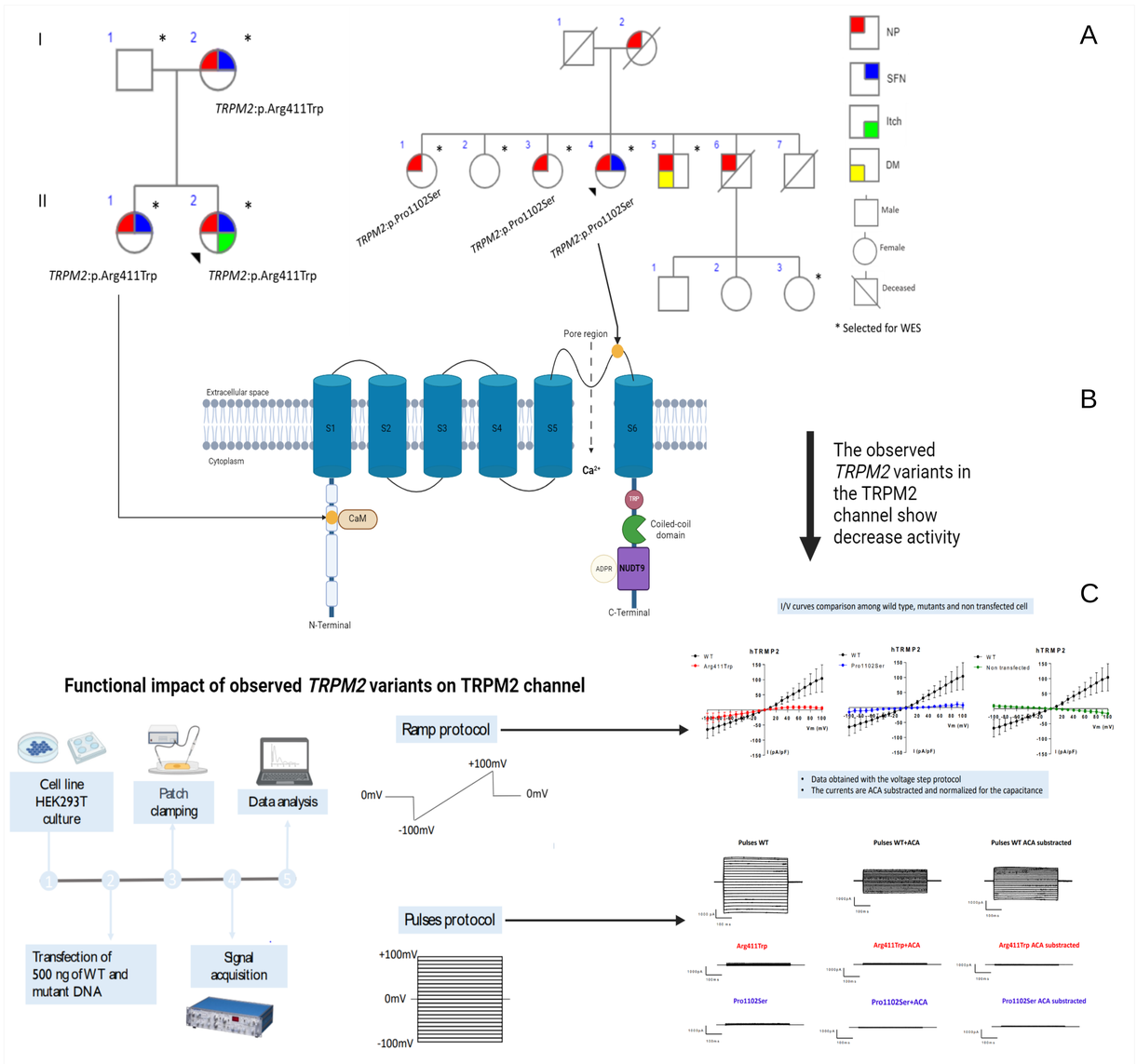

Figure 2: A) Pedigree of the two unrelated families with Small Fiber Neuropathy (SFN), Neuropathic Pain (NP), Itch and Diabetes Mellitus (DM). The ones with "*" were processed via Whole Exome Sequencing (WES). B) A pictorial representation of the TRPM2 channel consisting of six transmembrane regions (S1-S6) where N-terminal stands for the amino end of the protein and C-terminal is the carboxyl-side of the protein. The squares on the N-terminal represent the conserved region. Circle with TRP stands for transient receptor potential motif at the C-terminal along with coiled-coil domain and Nudix hydrolase (NUDT9) substrate attached with Adenosine diphosphate ribose (ADPR). The location of the genetic variants observed in both the families is displayed with yellow circles within the protein structure. This picture has been adapted from Nilius & Flockerzi, 2014b. C) Depicts the methodology adapted for whole-cell patch clamp study using both Ramp and Pulses protocol. The results from both the both protocols are displayed where WT stands for wild type and ACA stands for Anthranilic acid (ACA), a known TRPM2 channel blocker.

Conclusion

- This study reports two novel missense mutations in *TRPM2* segregating with painful SFN.
- Functional analysis revealed that both mutations affect the normal function of the TRPM2 channel, leading to a significant reduction in their currents.
- TRPM2 channel was previously linked to neuropathic pain in animal models but not in human genetic studies on neuropathic pain or SFN.
- These findings suggest that these mutations are likely causative and contribute to painful SFN development.
- Further investigations are warranted to elucidate the precise mechanisms by which these mutations lead to painful SFN and to speculate on TRPM2 as a potential pharmacological druggable target in future studies.

Future Studies

- Calcium Imaging: Measure intracellular calcium levels to assess TRPM2 channel activity and calcium influx in mutant vs. wild-type channels.
- Immunocytochemistry: Visualize the localization of TRPM2 in cells to see if mutations alter its distribution.
- Co-immunoprecipitation: Study interactions between TRPM2 and other proteins, like calmodulin, to understand if mutations disrupt regulatory mechanisms.

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