

Identifying TRPV4 antagonists with phenotypic screening from JUMP CP

Abstract

Our study on HMSN2C, a rare axonal neuropathy linked to TRPV4 gene mutations, utilized the JUMP CP dataset. Analysis yielded 3612 hits, with 87 found in a drug repurposing library. Literature review revealed three promising TRPV4 antagonists within this subset, offering potential therapeutic options for HMSN2C.

Overview

HMSN2C – hereditary motor and sensory neuropathy type IIC is a dominantly inherited axonal neuropathy with diaphragmatic and vocal cord paresis.

Known symptoms^{1,2} of HMSN2C include:

- Muscle weakness
- Vocal cord issues (paresis, hoarse voice, stridor)
- Sensory loss
- Respiratory problems
- Areflexia
- Skeletal abnormalities

Associated with various **mutations in the TRPV4 gene**.

The TRPV4 cation channel mediates **calcium influx in response to physical, chemical, and hormonal stimuli** in ciliated epithelial cells³.

It has various known functions:

- Responds to **changes in osmolarity**.
- Expresses in **tissues exposed to changing osmotic environments**⁴.
- **Regulates AQP5** abundance in response to hypotonic conditions⁵.

Results from animal studies suggest that **TRPV4 antagonism has therapeutic potential** in oedema, pain, gastrointestinal disorders, and lung diseases such as cough, bronchoconstriction, pulmonary hypertension, and acute lung injury⁶.

Our approach

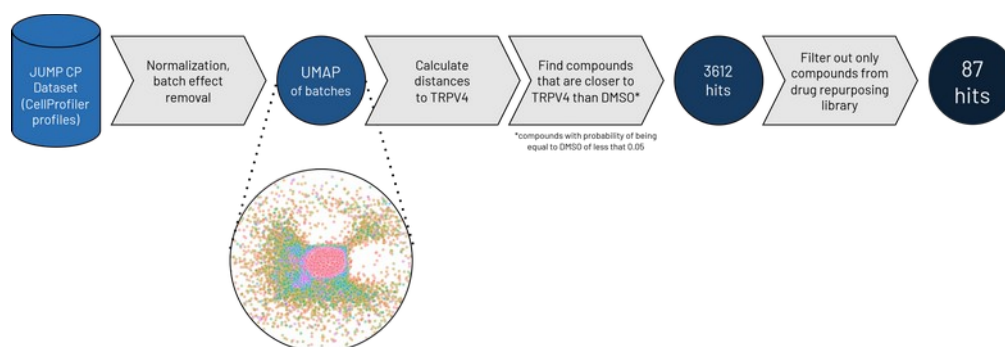


Figure 1: Analysis pipeline spanning from data normalization to hit identification.

Results

We calculated the distances between profiles of TRPV4 and all other compounds in the JUMP CP library. The hit threshold was defined by calculating a gaussian distribution of DMSO points and determining the probability of being smaller than the DMSO distribution. With our approach we were able to identify 3612 hits from the JUMP CP library with probability of being equal to DMSO 0.05 or less. Among these were 87 compounds from drug repurposing hub library⁷.

Then we conducted a literature review, revealing that certain compounds demonstrated promising potential as TRPV4 antagonists based on findings in existing literature.

GSK0660 is identified as a PPAR δ antagonist, with both PPAR δ and TRPV4 implicated in maintaining keratinocyte integrity. The authors describe the sequential regulation of TRPV4 $\rightarrow$ PPAR δ $\rightarrow$ AMPK, however, the specific impact of GSK0660 on TRPV4 remains a subject for further investigation⁸. We identified an enrichment of PPAR δ antagonists among the hits from the drug repurposing library with the odds ratio of 188:1.

Spermine is identified as a TRPV4 inhibitor^{9,10}. This inhibition is concentration- and voltage-dependent, reaching its maximum around the reversal potential, introducing a dynamic regulatory mechanism in excitable membranes during action potentials¹¹.

Meclofenamic acid has been established as a potent inhibitor of TRPV4. Notably, it exhibits a broader effect beyond TRPV4, also impacting other TRP channels. A separate study sheds light on mefenamic acid, a closely related compound, showcasing its selective inhibition of TRPM3-mediated calcium entry¹².

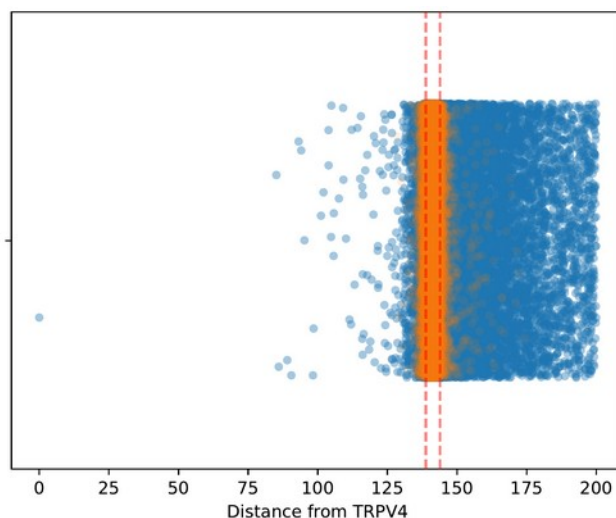


Figure 2: Distances from the gene of interest – TRPV4 (at 0) to all compounds from JUMP-CP dataset. Only distances less than 200 are plotted. Blue color represents compounds, orange color represents DMSO (negative control), dashed red lines represent the 0.05 probability thresholds of gaussian distribution of DMSO. The plot is one-dimensional with applied jitter in the vertical axis.

Conclusion

Several compounds in our dataset exhibit connections to TRPV4-related actions, such as calcium transport. Most of these compounds have not been previously described as TRPV4 modulators. Despite lacking specific identification, their potential impact on TRPV4-related processes suggests a broader range of compound-TRPV4 associations, warranting further investigation for novel insights into their roles in cellular activities linked to TRPV4.

Summary

- The JUMP CP dataset was utilized to identify compounds with potential antagonistic efficacy against TRPV4, the mutation of which causes HMSN2C, a rare neuropathy.
- The batch effect correction applied to multiple sources of datasets exhibited very good performance.
- The analysis pipeline was based on calculating the distances between profiles. This resulted in 3612 hits identified as compounds with smaller distance to TRPV4 knockdown than negative control.
- Hits were cross-referenced with a drug repurposing library, revealing 87 compounds.
- Further literature review identified three promising TRPV4 antagonists.

References

1. Dyck PJ, Litchy WJ, Minnerath S et al. Hereditary motor and sensory neuropathy with diaphragm and vocal cord paresis. *Annals of Neurology* 1994;35:608–15.
2. Chen D-H, Sul Y, Weiss M et al. CMT2C with vocal cord paresis associated with short stature and mutations in the TRPV4 gene. *Neurology* 2010;75:1968–75.
3. Chen H et al. A TRPV4 mutation caused CMT type 2C with scapuloperoneal muscular atrophy overlap syndrome and scapuloperoneal spinal muscular atrophy. *BMC Neurol* 2023;23:250.
4. Strotmann R, Harteneck C, Nunnenmacher K et al. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat Cell Biol* 2000;2:695–702.
5. Sidhaye VK, Güler AD, Schweitzer KS et al. Transient receptor potential vanilloid 4 regulates aquaporin-5 abundance under hypotonic conditions. *Proc Natl Acad Sci U S A* 2006;103:4747–52.
6. Grace MS, Bonvini SJ, Belvisi MG et al. Modulation of the TRPV4 ion channel as a therapeutic target for disease. *Pharmacology & Therapeutics* 2017;177:9–22.
7. Corsello SM, Bittker JA, Liu Z et al. The Drug Repurposing Hub: a next-generation drug library and information resource. *Nat Med* 2017;23:405–8.
8. Lee Y-J et al. Aster glehni Extract Containing Caffeoylquinic Compounds Protects Human Keratinocytes.... Evidence-Based Complementary and Alternative Medicine 2018;2018:e9616574.
9. Watanabe S, Kusama-Eguchi K, Kobayashi H et al. Estimation of polyamine binding to macromolecules and ATP in bovine lymphocytes and rat liver. *J Biol Chem* 1991;266:20803–9.
10. Bowie D, Mayer ML. Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. *Neuron* 1995;15:453–62.
11. Makshev G, Yuan P, Nichols CG. Blockade of TRPV channels by intracellular spermine. *J Gen Physiol* 2023;155:e202213273.
12. Klose C, Straub I, Riehle M et al. Fenamates as TRP channel blockers: mefenamic acid selectively blocks TRPM3. *Br J Pharmacol* 2011;162:1757–69.