

INTRODUCTION

Voltage-gated sodium channels (VGSC) are multimeric complexes with a critical role in action potential generation. While most subunits of the VGSC are transmembrane (TM) proteins, the β 1B subunit is a secreted splice variant of the SCN1B gene. This gene codes for a V-set domain containing protein of the immunoglobulin (Ig) superfamily, with a single Ig loop. β 1B is formed by retention of the intron upstream of the TM segment of the β 1 subunit. Because the retained intron lacks any functional domains, we hypothesize that its purpose is mainly the absence of the TM segment.

AIMS AND OBJECTIVES

This project seeks to investigate whether this is a conserved mechanism of forming soluble splice variants in other members of this protein family.

METHODS

A list of V-set domain containing proteins with a single Ig loop was obtained from the HUGO Gene Nomenclature Committee. Each protein was searched in the NCBI database and characterized by presence of sequenced mRNA splice variants, predicted subcellular location of the splice variants, and location of the splice sites. C-terminal domains of soluble isoforms were analyzed via BLAST for motifs and conservation among various animal species.

RESULTS

163 genes coding for V-set domain containing proteins with a single Ig loop were identified. Of these, 44.17% were found to contain only a single V-set domain, 76.39% of which have a single TM domain, and 47.22% had characterized splicing isoforms. 50% of single-TM proteins had predicted soluble splice variants, 94.12% of which were confirmed by TM domain prediction. Of the soluble isoforms, 11.11% contained motifs within the C-terminal region, and 40.74% were conserved only in simians.

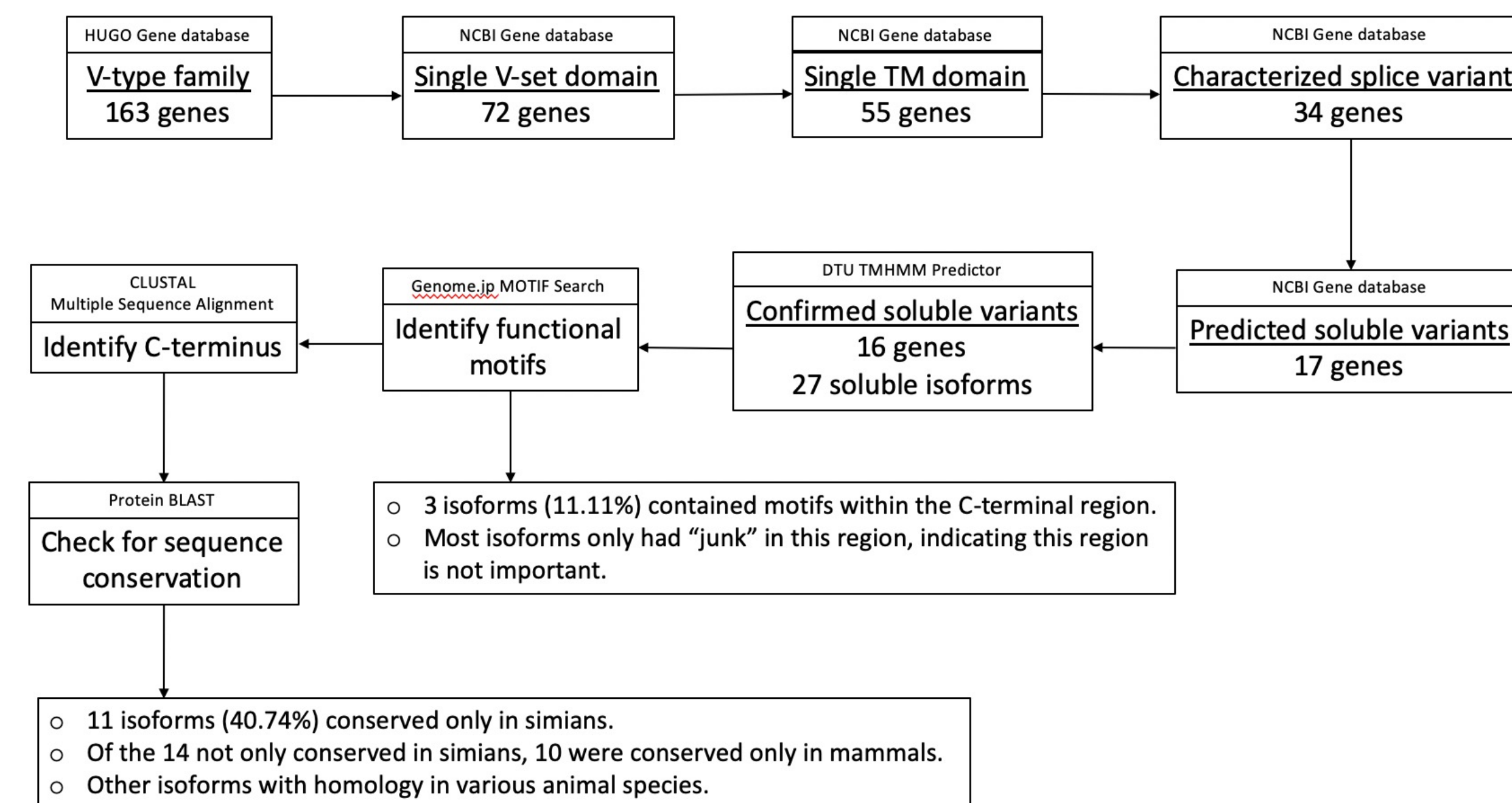


Figure 1. Refinement of results for the identification of conserved, soluble V-type domain-containing splice variants. Each large box represents the refined number of genes or method used to parse data. The program or database used for said step is listed in the box above.

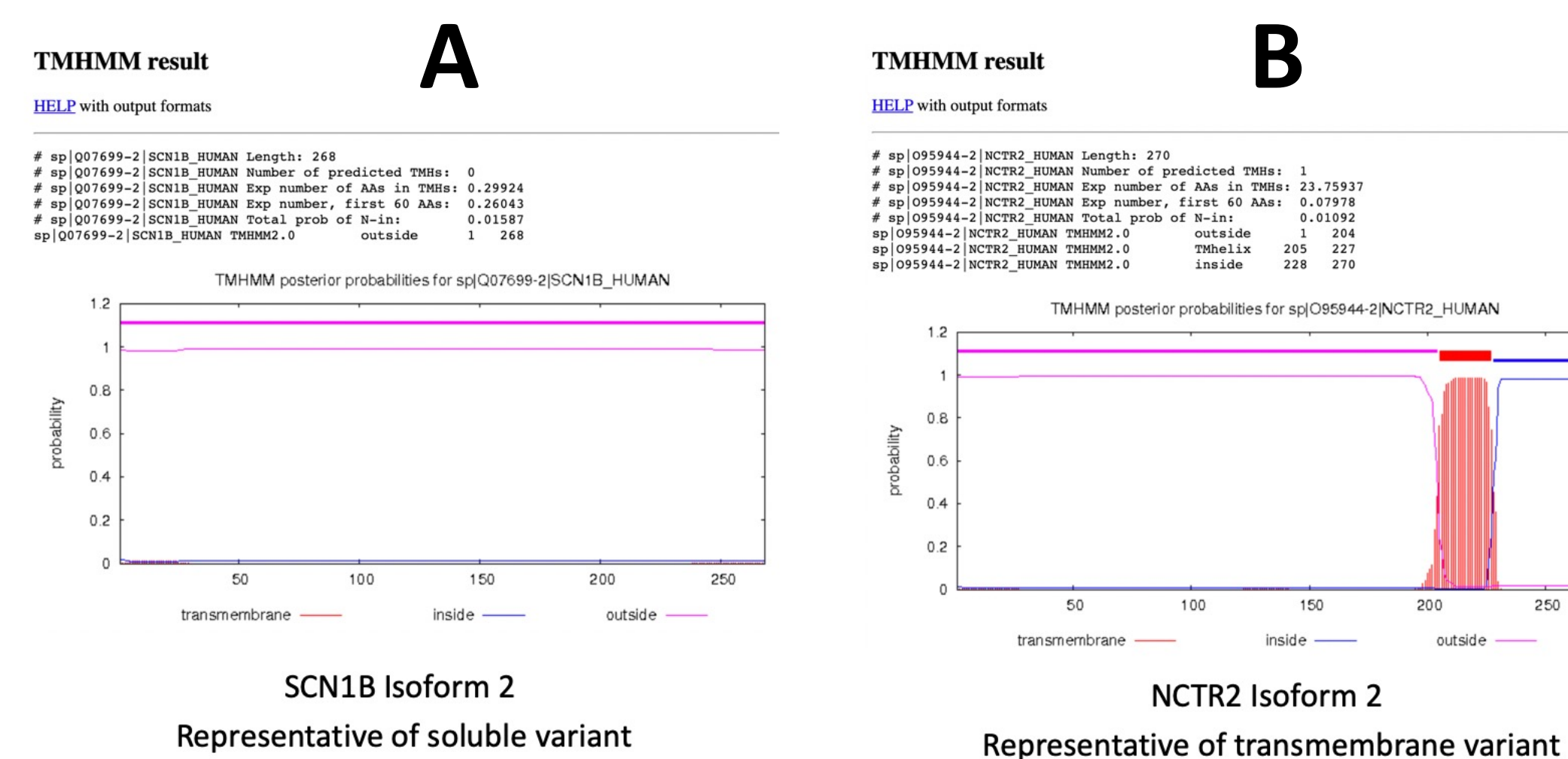


Figure 2. DTU TMHMM Predictor representative outputs for determination of membrane-bound versus soluble isoforms. (A) SCN1B isoform 2, also known as the β 1B subunit of the SCN1B gene, is a known soluble protein. (B) NCTR2 isoform 2, also known as Natural cytotoxicity triggering receptor 2 isoform 2, is a known membrane-bound protein with a single transmembrane domain.



Figure 3. CLUSTAL Multiple Sequence Alignment for determination of C-terminal sequence for BLAST. The native sequence (top) is compared to the sequence of the isoform (bottom).

CONCLUSIONS

Formation of soluble splice variants by retention of introns lacking functional domains is a recurring mechanism in for V-set domain containing proteins with a single Ig domain. This mechanism is highly conserved among mammals.

REFERENCES

1. Patino GA, Brackenbury WJ, Bao Y, et al. Voltage-gated Na⁺ channel β 1B: a secreted cell adhesion molecule involved in human epilepsy. *J Neurosci.* 2011;31(41):14577-14591. doi:10.1523/JNEUROSCI.0361-11.2011.
2. Qin N, D'Andrea MR, Lubin ML, Shafae N, Codd EE, Correa AM. Molecular cloning and functional expression of the human sodium channel β 1B subunit, a novel splicing variant of the β 1 subunit. *Eur J Biochem.* 2003;270(23):4762-4770. doi:10.1046/j.1432-1033.2003.03878.x
3. Dib-Hajj SD, Waxman SG. Genes encoding the β 1 subunit of voltage-dependent Na⁺ channel in rat, mouse and human contain conserved introns. *FEBS Lett.* 1995;377(3):485-488. doi:10.1016/0014-5793(95)01400-4
4. Oh Y, Waxman SG. The β 1 subunit mRNA of the rat brain Na⁺ channel is expressed in glial cells. *Proc Natl Acad Sci U S A.* 1994;91(21):9985-9989. doi:10.1073/pnas.91.21.9985

ACKNOWLEDGEMENTS

We would like to thank the EMBARK program and its directors for their support, guidance, and feedback throughout this process.