

Abstract

The brain-heart axis describes the close neural communication between the brain and the heart. Abnormal brain development can disrupt nerve signals and lead to cardiac disorders. This study uses bioinformatic tools to analyse human PHOX2B, a key transcription factor controlling autonomic neuron development. We performed transmembrane prediction, cross-species sequence alignment, protein-protein interaction analysis and CRISPR editing strategy design. Our results show PHOX2B is intracellular and highly conserved across species. It cooperates with RET, GDNF and ASCL1 to regulate neurons within the brain-heart axis. Since this research relies only on database prediction without wet-lab experiments, further laboratory validation is required. This work provides theoretical support and clear directions for future experimental studies.

The relationship between the brain development and the heart function in the context of PHOX2B mediated signalling

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Introduction

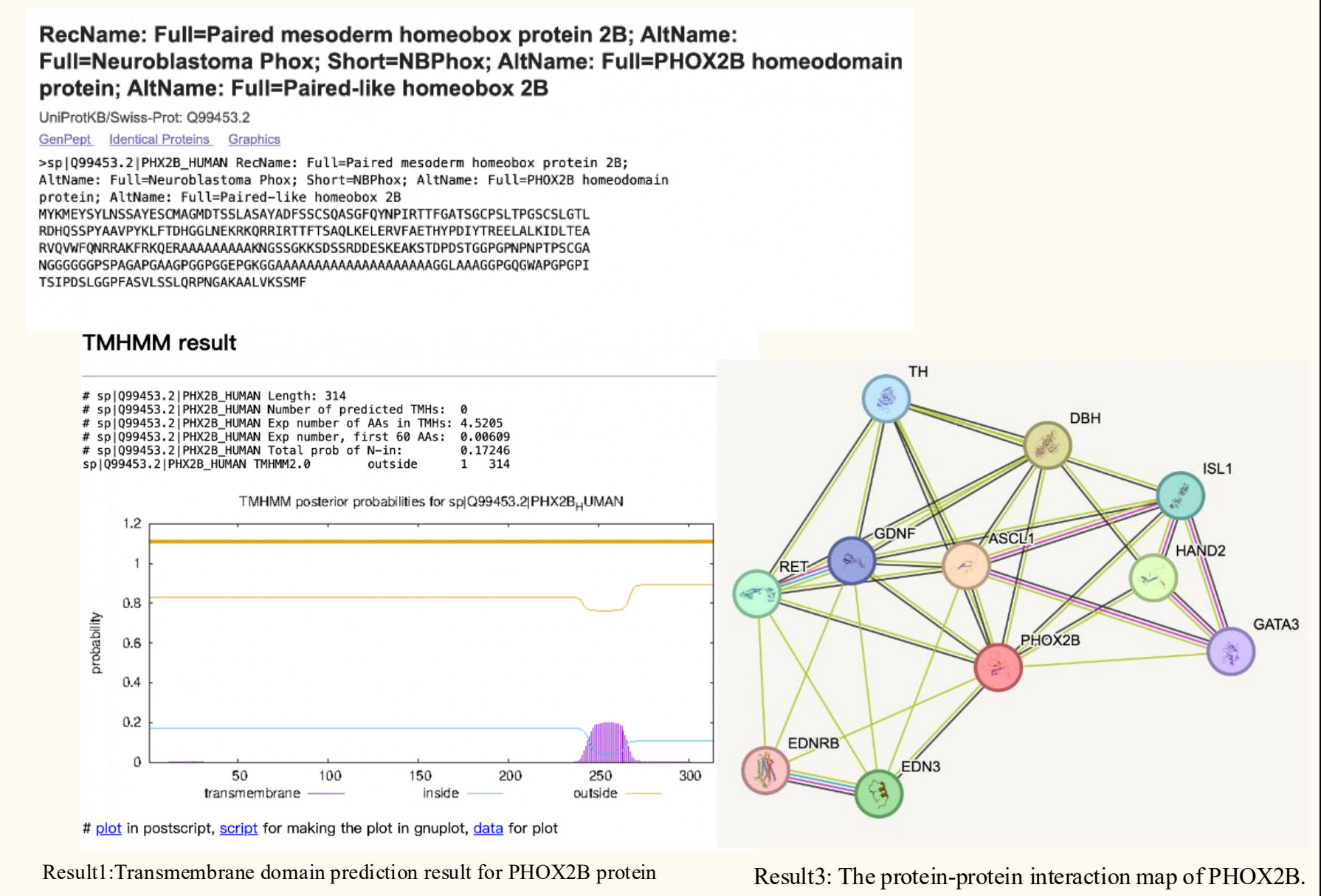
The brain-heart axis mediates critical bidirectional neural signalling between the brain and the heart[1]. Defects in autonomic nervous system development disrupt cardiac regulation and contribute to severe neurocardiac diseases. Previous studies have established that the transcription-factor-encoding gene PHOX2B acts as a master regulator for autonomic neuron maturation; pathogenic PHOX2B mutations are linked to disorders including Congenital Central Hypoventilation Syndrome[2]. However, the detailed protein features, evolutionary conservation and full interaction network of human PHOX2B remain incompletely characterised. This study aims to analyse human PHOX2B via multiple bioinformatic workflows and design a CRISPR-Cas9 editing framework, to provide theoretical foundations for future wet-lab investigation of PHOX2B-driven brain-heart regulatory mechanisms.

Methods

MethodsHuman PHOX2B protein sequence (accession NP_003915.2, 314 amino-acids) was downloaded in FASTA format from the NCBI Gene database. Genomic metadata including gene ID (ENSG00000109132) was retrieved from Ensembl.Trans-membrane domain prediction was performed on the TMHMM web server using the full-length PHOX2B amino-acid sequence. Cross-species multiple-sequence alignment was run via Clustal Omega using vertebrate ortholog sequences. The STRING database was used to construct the human PHOX2B protein-protein interaction network.CRISPR-Finder screened candidate sgRNA targets for PHOX2B. Off-target scores were prioritised for sgRNA selection. SnapGene software annotated gene domains and visualised CRISPR-Cas9 cutting sites. Proposed post-editing validation includes PCR amplification, Sanger sequencing and Western blot analysis.

Results

ResultsTransmembrane-domain prediction demonstrates that human PHOX2B contains no transmembrane helices, confirming its identity as an intracellular nuclear transcription factor.Cross-species multiple-sequence alignment reveals high evolutionary conservation of PHOX2B protein across vertebrate species, indicating functionally critical amino-acid regions.STRING protein-protein interaction analysis identifies key partners including RET, GDNF, ASCL1, DBH and HAND2, which jointly participate in autonomic neuron development.Candidate sgRNA sequences for PHOX2B CRISPR-Cas9 editing were selected with low off-target scores, providing potential editing sites for future mutagenesis experiments.



Discussion

My analysis supports the result that human PHOX2B contains no transmembrane helices, confirming its identity as an intracellular nuclear transcription factor. Findings from my work show high evolutionary conservation of PHOX2B protein across vertebrate species, indicating functionally critical amino-acid regions. The STRING protein-protein interaction analysis carried out in my study identifies key partners including RET, GDNF, ASCL1, DBH and HAND2, which jointly participate in autonomic neuron development.All these results come from computational prediction rather than wet-lab experiments. Candidate sgRNA sequences for PHOX2B CRISPR-Cas9 editing, which I selected with low off-target scores, provide potential editing sites for future mutagenesis experiments to further explore the functions of PHOX2B

Conclusion

In summary, my analyses demonstrate that human PHOX2B contains no transmembrane helices and acts as an intracellular nuclear transcription factor. Cross-species comparison within my project reveals high evolutionary conservation across vertebrate species, marking functionally critical amino-acid regions. Key interaction partners including RET, GDNF, ASCL1, DBH and HAND2, which jointly participate in autonomic neuron development, have been identified through my work. Candidate sgRNA sequences with low off-target scores provide potential editing sites for future mutagenesis experiments. My work provides computational findings for further research on PHOX2B.

References

- [1] Valenza, Gaetano, Zoran Matić, and Vincenzo Catrambone. 2025. "The Brain-Heart Axis: Integrative Cooperation of Neural, Mechanical and Biochemical Pathways." *Nature Reviews. Cardiology* 22 (8): 537–50. <https://doi.org/10.1038/s41569-025-01140-3>.
- [2] Windels, Mei-Lan, Fleur Cordier, Jo Van Dorpe, Liesbeth Ferdinande, and David Creytens. 2024. "PHOX2B: A Diagnostic Cornerstone in Neurocristopathies and Neuroblastomas." *Journal of Clinical Pathology* 77 (6): 378–82. <https://doi.org/10.1136/jcp-2023-209047>.