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RESEARCH ARTICLE

Single-Cell Transcriptomic Analysis of Human Spinal Cords Elucidates Cell-Type-Specific Pathomechanisms in HNRNPH2-Related Neurodevelopmental Disorder

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Abstract

HNRNPH2-Related Neurodevelopmental Disorder (HNRNPH2-RNDD) is an ultra-rare, X-linked condition characterized by motor impairments, intellectual disability, and global developmental delay. HNRNPH2 is a ubiquitously expressed protein, and the mechanism by which its mutations cause profound neurodevelopmental and motor dysfunction remains unclear. To investigate the cellular basis of these impairments, we performed an integrative single-cell RNA sequencing (scRNA-seq) analysis of adult human spinal cord datasets. In addition, CellChat, a computational framework for inferring intercellular communication networks, was performed to delineate cell-cell communication associated molecular mechanism of motor impairment caused by HNRNPH2-RNDD. Using a dataset of 24,190 cells, we identified cell-type-specific expression patterns of HNRNPH2 gene and mapped ligand-receptor signaling pathways potentially affected by the gene dysfunction. HNRNPH2 gene expression was enriched in non-central nervous system cell populations such as endothelial cells, pericytes, and ependymal cells. The CellChat analysis revealed robust outgoing communication from these HNRNPH2-expressing cell types to neurons, astrocytes, and oligodendrocytes via key neurodevelopmental pathways, including NRG3, NRXN1, and NCAM1. Correlation analyses further demonstrated statistically significant associations between HNRNPH2 gene expression and these signaling genes, suggesting a crucial role for HNRNPH2 in maintaining communication networks essential for neuronal maturation, synaptic stability, and motor system development. The bulk RNA-seq analysis with HNRNPH2-RNDD models also suggested a correlation between HNRNPH2 and the identified neurodevelopmental factors. These findings support a novel model in which disruption of HNRNPH2 in non-neuronal spinal cord cells impairs critical intercellular signaling and may contribute to the complex motor and developmental phenotypes observed in HNRNPH2-RNDD. This work establishes a foundation for future disease-specific scRNA-seq studies and highlights potential therapeutic signaling pathways for targeted intervention.

Introduction

The ultra-rare, X-linked, heterogeneous nuclear ribonucleoprotein H2 (HNRNPH2)-Related Neurodevelopmental Disorder (HNRNPH2-RNDD) is caused by de novo pathogenic missense variants in the HNRNPH2 gene, which encodes the RNA-binding protein HNRNPH2 [1-3]. The disorder arises from pathogenic de novo missense variants in the HNRNPH2 gene, located on the long arm of the X chromosome at locus Xq22 (1-3). HNRNPH2 is a member of the large and ubiquitously expressed HNRNP family of RNA-binding proteins, the fundamental regulators of RNA metabolism that play critical roles at multiple stages of a messenger RNA's life cycle [2,4]. Recently, several HNRNP family members have been implicated in multiple neurodevelopmental disorders [4]. HNRNPH2 and its family members are primarily localized to the nucleus, playing roles in pre-messenger RNA (pre-mRNA) transcripts and forming large ribonucleoprotein complexes. They act as crucial modulators of post-transcriptional gene regulation that influence essential processes such as alternative splicing, polyadenylation, mRNA stability, and the subsequent transport of mature mRNA from the nucleus to the cytoplasm for translation [2,4,5].

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Likely associated with its ubiquitous expressions and multiple functions, the HNRNPH2-RNDD causes patients with a broad range of pathological features. Patients with HNRNPH2-RNDD are non-ambulatory, nonverbal, with dysmorphic craniofacial features [3,6,7]. Intellectual disability, epilepsy, autism, and oral stimulation are also present in the patients, leading to their limited independence [3,6,7]. Nearly all patients exhibit global developmental delay and intellectual disability [1,3,6]. Language impairment is profound, as most individuals are non-verbal or minimally verbal, relying on alternative communication devices or basic gestures, which severely limit their ability to express pain, hunger, or emotion. Motor dysfunction is also profound, as hypotonia (low muscle tone) is universal in infancy and dystonia as the child ages [1,3,6]. These result in delayed motor milestones, as many children do not walk until age five and often require orthotics, wheelchairs, or gait trainers [3]. In addition, psychiatric and behavioral comorbidities are the most distressing aspect for families, exhibiting autism spectrum, sensory processing disorders, and self-injurious behaviors [8]. Moreover, systemic comorbidities such as disorders in multiple organ systems, rapidly progressive scoliosis, hip dysplasia, and gastrointestinal dysfunction. These clinical presentations of HNRNPH2-RNDD are characterized by a spectrum of severity that imposes a lifelong, 24-hour care burden on families [1,3,8].

Despite the significant healthcare burden of the family impacted by HNRNPH2-RNDD, there is no robust research and development (R&D) toward developing therapeutics for the disease. Identified in 2016, HNRNPH2-RNDD is an ultra-rare disease, currently affecting between 145 and 200 individuals worldwide. The small number of patients places the disease well below the commercial threshold required to attract traditional pharmaceutical investment or venture capital. Unlike other rare diseases, such as Spinal Muscular Atrophy (SMA) and Cystic Fibrosis (CF), which have multi-billion-dollar markets and transformative therapies, HNRNPH2-RNDD exists in a capital vacuum [9]. The pharmaceutical industry and institutional investors most likely view the patient population as insufficient to generate returns on the immense risk-adjusted costs of R&D, rendering the market unfeasible [9]. Consequently, the burden of funding basic science, natural history studies, and even the administrative costs of clinical trials has shifted entirely to patient families and small charitable foundations, such as the Yellow Brick Road Project (YBRP) and the HNRNP Family Foundation. This aspect further underscores the need for innovative research to revolutionize current treatment for HNRNPH2-RNDD.

Although there has been promising research progress, we still do not have any FDA approved therapies available for HNRNPH2-RNDD, except for symptoms management [10,11]. Current therapeutic strategies in development include allele specific and non-specific antisense oligonucleotides (ASOs) - short, double-stranded DNA or RNA strands designed to selectively bind to specific messenger RNA (mRNA) to control protein production - to downregulate or knock out the mutated HNRNPH2 genes [12,13]. Currently, a pilot n-of-3 clinical trial is undergoing at Columbia University Irving Medical Center in collaboration with the n-Lorem Foundation using non-allele specific ASO. Although the ongoing approaches are valid, the remaining challenges for developing effective therapeutics include our limited understanding of HNRNPH2, especially for the specific mechanism of how HNRNPH2 mutations cause a wide spectrum of neurodevelopmental disorders [10]. Thus, delineating the molecular mechanism associated with HNRNPH2-RNDD may support the ongoing efforts to develop a highly effective and specific therapy [10]. In this study, as motor impairment is a crucial aspect of HNRNPH2-RNDD, we explored the potential pathomechanisms of HNRNPH2-RNDD-caused motor dysfunctions using state-of-the-art single-cell RNA sequencing (scRNA-seq). Our central hypothesis is that scRNA-seq analysis will lead us to identify the specific molecular mechanisms responsible for motor function impairments in HNRNPH2-RNDD. Targeting motor impairment is essential, as it is one of the hallmarks of HNRNPH2-RNDD, manifesting as a complex and multifaceted motor impairment disorder rather than a singular deficit. Moreover, the molecular mechanism underlying the complex spectrum of motor dysfunction in HNRNPH2-RNDD remains largely unknown. To understand the cellular basis of the profound motor deficits in HNRNPH2-RNDD, we selected the healthy adult spinal cord single cell atlas, as the anatomical structure where motor control is executed and the primary information superhighway of the motor system, relaying nerve signals between the brain and the rest of the body to control movement, sensation (touch, pain, temp), and involuntary functions [14].

Single-cell RNA sequencing (scRNA-seq) has become a transformative methodology for investigating complex biological systems at unprecedented resolution [14-16]. By enabling the isolation and transcriptomic profiling of individual cells, scRNA-seq offers a detailed view of gene expression heterogeneity and supports the systematic classification of thousands of cells into molecularly distinct populations. Since its introduction in 2009, the technology has generated extensive insights across diverse organisms, including humans, animal models, and plants, facilitating the construction of high-resolution cellular atlases that serve as essential references for understanding tissue organization, cellular interactions, and the molecular basis of health and disease [17,18]. The utility of scRNA-seq has been particularly evident in neuroscience, where its application to the developing and adult human spinal

cord has delineated its complex cellular landscape, identified numerous neuronal subtypes, and traced their developmental trajectories, thereby establishing a foundational framework for interpreting spinal cord biology [19-22]. Beyond characterizing normal tissue architecture, scRNA-seq provides a powerful strategy for studying disease mechanisms. By analyzing transcriptional profiles across all constituent cell types within affected tissues, researchers can identify the specific populations most perturbed by pathogenic variants, define cell-type-specific vulnerabilities, and map the molecular pathways disrupted in those cells [19-22]. This level of resolution is especially critical for disorders arising from dysfunction in ubiquitously expressed genes, such as HNRNPH2-RNDD, where a key question concerns why a broadly expressed protein leads to selective phenotypic manifestations. scRNA-seq may elucidate which cell types are particularly dependent on the gene's function and thus more susceptible to its disruption.

In addition, this study implemented an emerging analysis tool, CellChat [23,24], integrated with scRNA-seq. CellChat is a computational framework developed to quantitatively infer and analyze intercellular communication networks from single-cell transcriptomic data. It estimates the probability of signaling between cell populations using a mass-action–inspired model that accounts for core ligand–receptor interactions, including multisubunit complexes, as well as modulation by cofactors. The platform predicts primary signaling inputs and outputs for each cell type and characterizes how these signals coordinate cellular functions through network-based analyses and machine-learning-driven pattern recognition. CellChat performs systematic and comparative analyses of intercellular communication, providing a variety of quantitative metrics to assess signaling strength, network hierarchy, and information flow across cell types. The latest version, CellChat v2, expands these functions by adding further-enriched ligand-receptor database providing functional annotations, additional tools for comparative analysis, and an interactive visualization interface. Together, these features allow researchers to comprehensively map and interpret complex cell-cell communication networks in an accessible and reproducible framework. Our scRNA-seq and CellChat analysis identified the specific cell types highly expressing HNRNPH2 gene in healthy adult spinal cord and their roles in communication with the primary central nervous system (CNS) cells. The discovered cell-cell signaling pathway associated with HNRNPH2-expressing cells are potentially involved in HNRNPH2-RNDD and the consequent motor function impairments. Thus, our findings reported below may have significant implications in the development of novel therapeutic targets to address the motor function impairments in HNRNPH2-RNDD patients. This study also provides a strong foundation for understanding the biological roles of HNRNPH2 and how HNRNPH2 mutations may lead to disrupted cell-cell communication.

Methods

Single-cell RNA sequencing (scRNA-seq) dataset from healthy, adult human spinal cord tissues were obtained from the Chan–Zuckerberg Foundation's CELLxGENE database, yielding a dataset of 24,190 cells for analysis [25,26]. The dataset includes the integrated single cell transcriptomic atlas of spinal cords from 20 human donors (age: 30 - 75; 10 males and 10 females) [27]. All computational processing and visualization were performed using ICARUS version 3.0, an interactive web-based R analysis platform [28], together with RStudio version 5.1. The cell count matrix and annotation data were loaded from an h5ad file, from which the Seurat objects were generated using Seurat (version 5.2.1). Quality control steps entailed filtering out cells with fewer than 200 or more than 6,000 detected features and with greater than 20% mitochondrial gene expression. Gene expression data were then globally normalized, and the top 2,000 variable genes were identified using a variance-stabilizing transformation. Scaled data were subjected to principal component analysis (PCA). The number of principal components used in downstream analyses was determined via the elbow plot by identifying the inflection point at which additional principal components contributed only marginally to the variance. Doublet detection was performed using DoubletFinder (version 2.0.4) with an estimated doublet rate of approximately 7.5%, and only cells classified as singlets were retained.

Following normalization and filtering, clustering was performed using the optimal number of PCA components, which was determined from the elbow plot. Uniform Manifold Approximation and Projection (UMAP) was used to identify transcriptionally distinct cell clusters across the dataset. The labeling of identified cell clusters was conducted utilizing the annotation data from CELLxGENE. Following cluster identification, gene expression profiling was conducted to determine which cell populations expressed HNRNPH2 at high levels. To investigate potential mechanisms of cellular interaction associated with HNRNPH2, we performed CellChat analysis, a computational framework that infers intercellular communication networks by predicting ligand–receptor signaling pathways between cell types. A dedicated Seurat object was constructed for CellChat analysis using CellChat (version 1.6.1) and associated packages (circlize version 0.4.16, grid version 4.4.2, and Matrix version 1.7.3), with a human-specific CellChat database. Custom visualization functions were developed to generate two types of plots: a circular network plot and a chord diagram. The circular network plot displays the global cell-cell communication landscape, where vertex sizes indicate cell population sizes and edge weights reflect the strength of

communication probabilities. The chord diagram is designed to illustrate specific signaling interactions, highlighting the top pathways based on interaction scores or selected pathways of interest. In these chord diagrams, chord thickness denotes the communication strength between cell types, offering insights into the key ligand–receptor mediated interactions. Heatmaps were generated to quantify incoming and outgoing signaling strengths across these cell populations. Additionally, correlation analyses were conducted to assess the relationship between HNRNPH2 expression and key communication-related genes associated with neuronal development and motor function, including neuregulin 3 (NRG3), neurexin-1 (NRXN1), neural cell adhesion molecule 1 (NCAM1), and neuronal growth regulator 1 (NEGR1). These combined approaches enabled a comprehensive examination of cell types expressing HNRNPH2 and their potential communication with neural populations within the human spinal cord.

To evaluate the identified correlation between the neuronal factors and HNRNPH2, we performed a comparative analysis for those gene expressions between wild-type and HNRNPH2 knockout (KO) and HNRNPH2 mutation inserted into human induced pluripotent stem cell-derived neurons or Hnrnph2 mutations into mouse [10]. Bulk RNA-seq data of human iPSC (GSE226525) and mouse cortex (GSE226526) were examined for quantitative transcriptomics per million (TPM), followed by quantitative comparison between wild-type (WT), KO, and mutations at R206W, P209L, or R206Q (human iPSC only).

Results

Specific cell types highly expressing HNRNPH2

Our scRNA-seq analysis identified multiple transcriptionally distinct cell clusters within human spinal cord tissue, spanning both neuronal and non-neuronal lineages (Figure 1A). The identified cell clusters included oligodendrocyte, astrocyte, oligodendrocyte precursor cell, central nervous system macrophage, neuron, leukocyte, endothelial cell, pericyte, and ependymal cell (Figure 1A). Gene expression profiling revealed that HNRNPH2 expression was enriched primarily in non-neuronal populations, including endothelial cells, pericytes, and ependymal cells (Figure 1B), confirming the localization of HNRNPH2 within specific supportive and vascular-associated cell types. The gene expression assay showed that over 25% of endothelial cells, pericytes, and ependymal cells highly express HNRNPH2 (Figure 1B).

CellChat elucidated cell-cell communications between HNRNPH2-expressing cells and other CNS cells

CellChat analysis revealed active cell-cell communications spinal cord cell populations, with particularly strong signaling connections between the HNRNPH2-expressing non-neuronal cells and central nervous system (CNS) cell types (Figure 2A and 2B).

Heatmaps showed the intensities of outgoing signal and incoming signal for each type of cell (Figure 3). The strong outgoing signaling pathway from endothelial cells, pericytes, and ependymal cells include but not limited to NEGR, NCAM, NRG, NRXN, TAG, PROS, and GAS (Figure 3). From the strong outgoing signal, we identified several pathways, NEGR, NCAM, NRG, and NRXN, showing notable strength both in outgoing and incoming signals (Figure 3). We selected these four signaling pathways for the further analyses given their robust outgoing signals from endothelial cells, pericytes, and ependymal cells, targeting a range of CNS cell populations (Figure 3), and their reported functions in neurodevelopment and motor function in spinal cords [29,30].

HNRNPH2-expressing cells may regulate neuronal development, function, and homeostasis via cell-cell communications

A bubble plot of the selected ligand-receptor pairs in NEGR, NCAM, NRG, and NRXN signaling pathways confirmed the statistically significant, robust signaling emanating from endothelial, pericytes, and ependymal cells to oligodendrocytes, astrocytes, and neurons (Figure 4A). In particular, NRXN1 signal was robust in communication from endothelial cells, pericytes, and ependymal cells to neurons (Figure 4A). NRG3 signaling was highly expressed in cell-cell communication of endothelial cell-to-astrocyte & neuron, pericyte-to-astrocyte & neuron, and ependymal cell-to-astrocyte & neuron (Figure 4A). Similarly, robust NEGR1 signaling was identified in endothelial cell & pericyte-to-oligodendrocyte and ependymal cell-to-oligodendrocyte (Figure 4A). Strong NCAM1 signal was observed in the communication of endothelial cell-to-oligodendrocyte & astrocyte and pericyte-to-oligodendrocyte & astrocyte (Figure 4A). In summary, the CellChat analysis suggests that HNRNPH2-expressing non-neuronal cells communicate to CNS cells through NEGR, NCAM, NRG, NRXN signaling pathways (Figure 4B).

Pearson correlation analysis showed the statistically significant correlation between HNRNPH2 expression and NRG3, NRXN1, and NCAM1 expression ($p < 0.0001$). This finding strongly suggests the role of HNRNPH2 in cell-cell communication mediated by NRG3, NRXN1, and NCAM1 signaling (Figure 5A-5C). However, NEGR1 signal showed no statistically significant correlation with HNRNPH2 expression (Figure 5D). Together, these results suggest that HNRNPH2-expressing cell types participate in communication pathways to CNS cells essential for neural development, maintenance, and motor system function.

Bulk RNA-seq analysis for mouse cortex showed that Hnrnph2 KO or mutation at R206W do not alter

expressions of Nrg3, Nrxn1 or Ncam1 (Figure 6A-6C). In contrast, Nrg3, Nrxn1 and Ncam1 expressions were significantly reduced in the mouse cortex with Hnrnph2 mutation at P209L (Figure 6A-6C). In mouse Hnrnph2 disease model, both R206W and P209L mutations cause neurodevelopmental disorders, but P209L generally exhibit more severe neurological and motor impairments [10]. Thus, the significant reduction in Nrg3, Nrxn1, and Ncam1 in P209L mutation is suggested to be associated with Hnrnph2-related disorders. In human iPSC-derived neurons, HNRNPH2 KO and mutations significantly increased NRG3, NRXN1, and NCAM1 expressions, with dramatic increases with mutations at R206W and P209L (Figure 6D-6F). In addition, Pearson's correlation analysis confirmed the statistically significant correlation between HNRNPH2/Hnrnph2 and the NRG3/Nrg3, NRNX1/Nrnx1, and NCAM1/Ncam1 ($p < 0.001$) (Figure 6G,6H). Although the results of mutations appear to be toward different direction in mouse and human models, these data likely confirm the close link

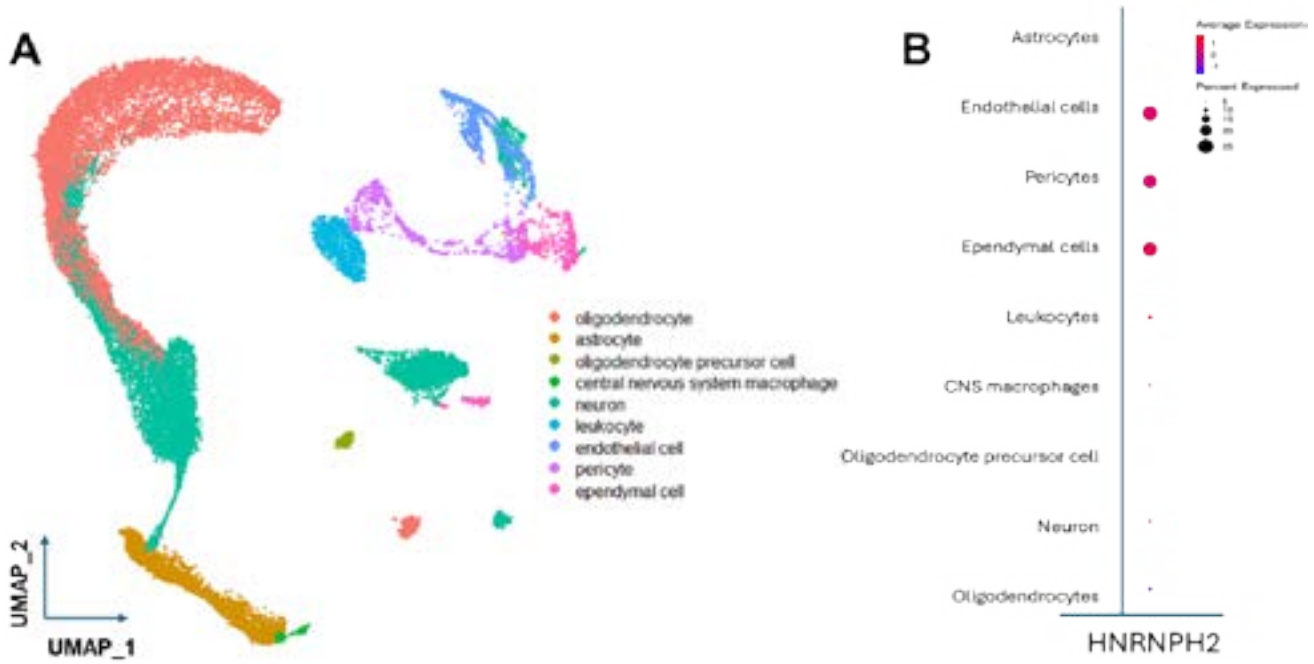

Figure 1: (A): UMAP identified multiple cell clusters in human spinal cords; (B): Gene expression profiles show cells types highly expressing HNRNPH2.

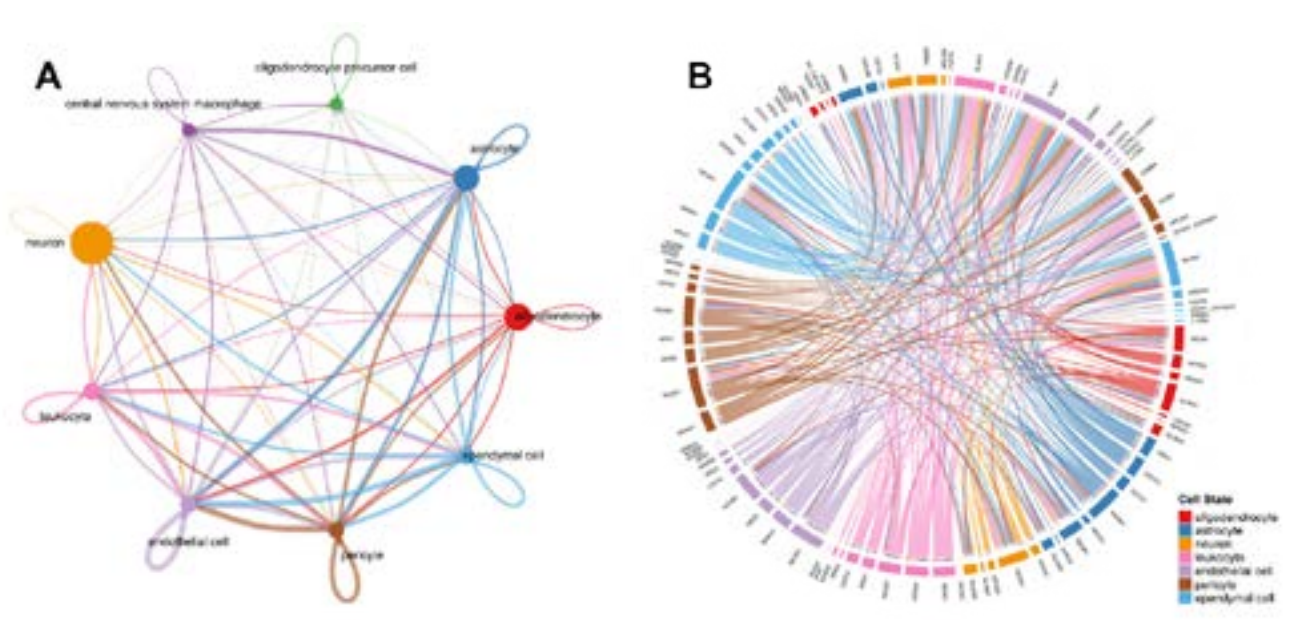

Figure 2: CellChat analysis shows active interactions between different cell types: (A): A Circle plot for the intensity of cell-cell communication; (B): and a Chord plot for signaling involved in cell-cell communication between specific cell type pairs. Strong cell-cell communications are observed between non-neuronal cells and CNS cells.

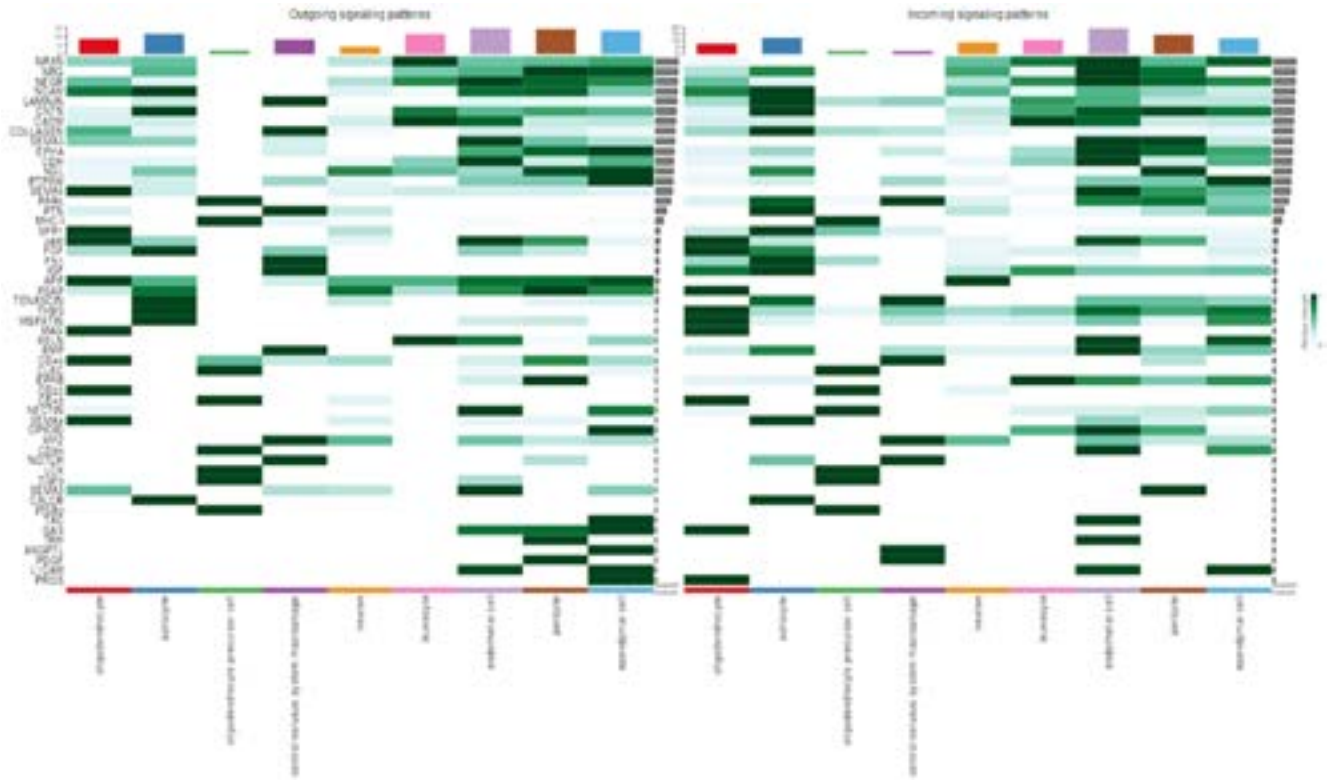

Figure 3: Heatmaps showing the strength of outgoing and incoming signals per cell type. Robust outgoing signals are observed in non-neuronal cells, with strong incoming signals to CNS cells.

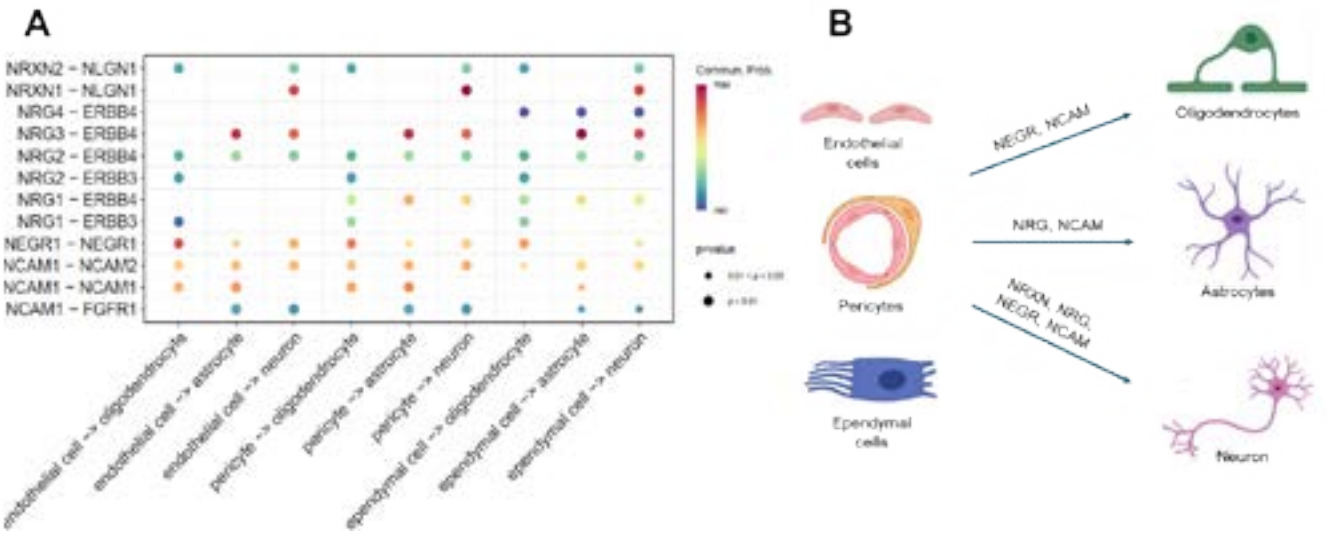

Figure 4: (A): Bubble plot shows signaling ligand-receptor pairs significantly activated between HNRNPH2-expressing cells (endothelial cells, pericytes, and ependymal cells) and other types of cells in the spinal cord; (B): CellChat analysis data collectively suggest that HNRNPH2-expressing cells send out signals to CNS cells, associated with motor system development and protection.

between HNRNPH2/Hnrnph2 and the neuronal factors identified as cell-cell communication mediator from our CellChat analysis.

Discussion

The findings from this study support a model in which HNRNPH2-expressing endothelial cells, pericytes, and ependymal cells influence neuronal development, homeostasis and motor system function through communication signals mediated by pathways, such as NRG, NRXN, and NCAM. These signaling systems are well established as essential regulators of neuronal maturation, synaptic stability, and axon guidance (29-31), thus aligning with the types of motor and developmental impairments observed in HNRNPH2-RNDD. The strong correlations identified between HNRNPH2 expression and NRG3, NRXN1, and NCAM1 expressions further support the hypothesis that

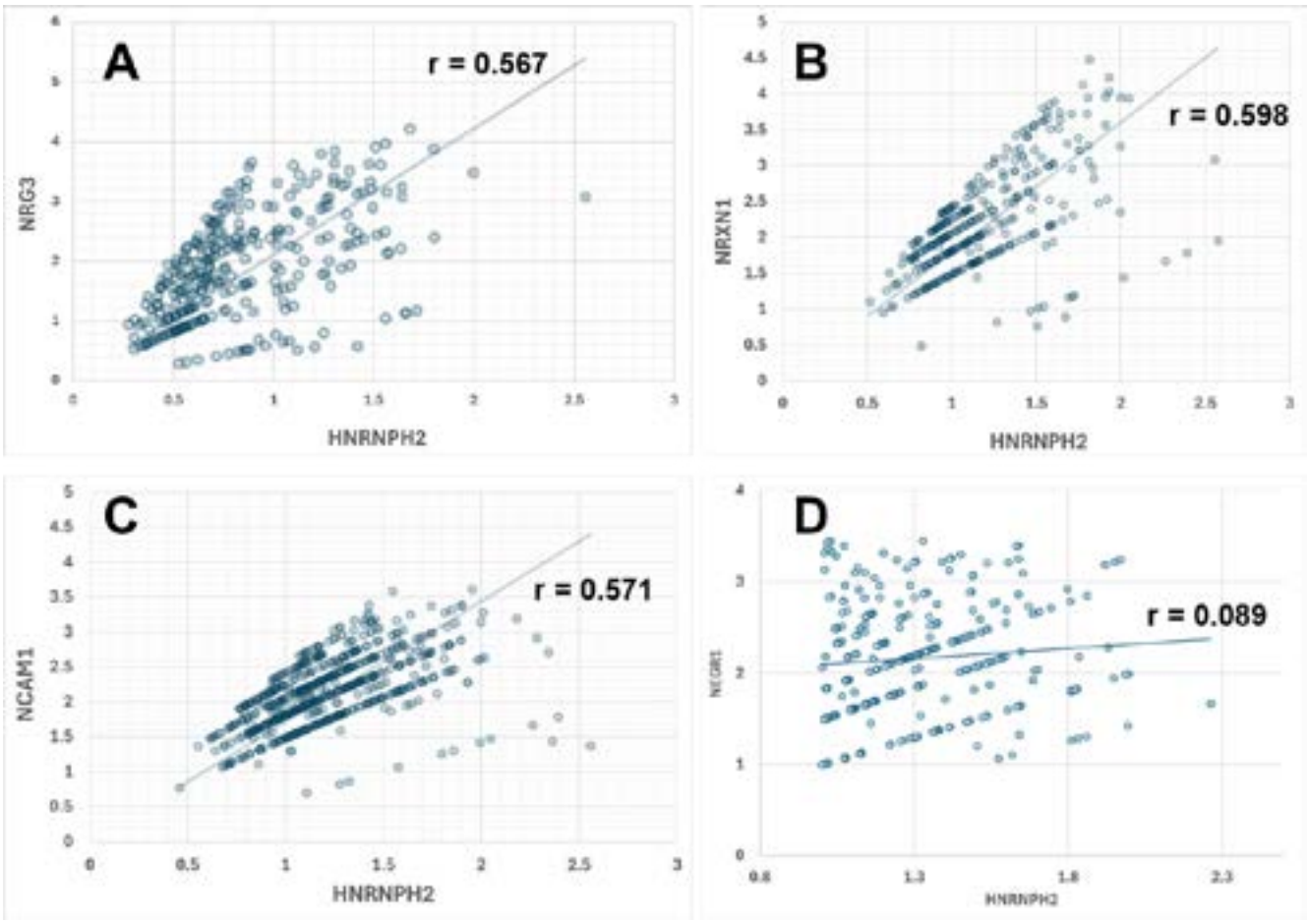

Figure 5: (A-C): HNRNPH2 expression is statistically correlated with the expression of the NRG3, NRXN1, and NCAM1 involved in neuronal functions and development (r: Pearson correlation coefficient, p<0.0001). NEGR1 shows no statistically significant correlation with HNRNPH2 expression (D) (p = 0.1165).

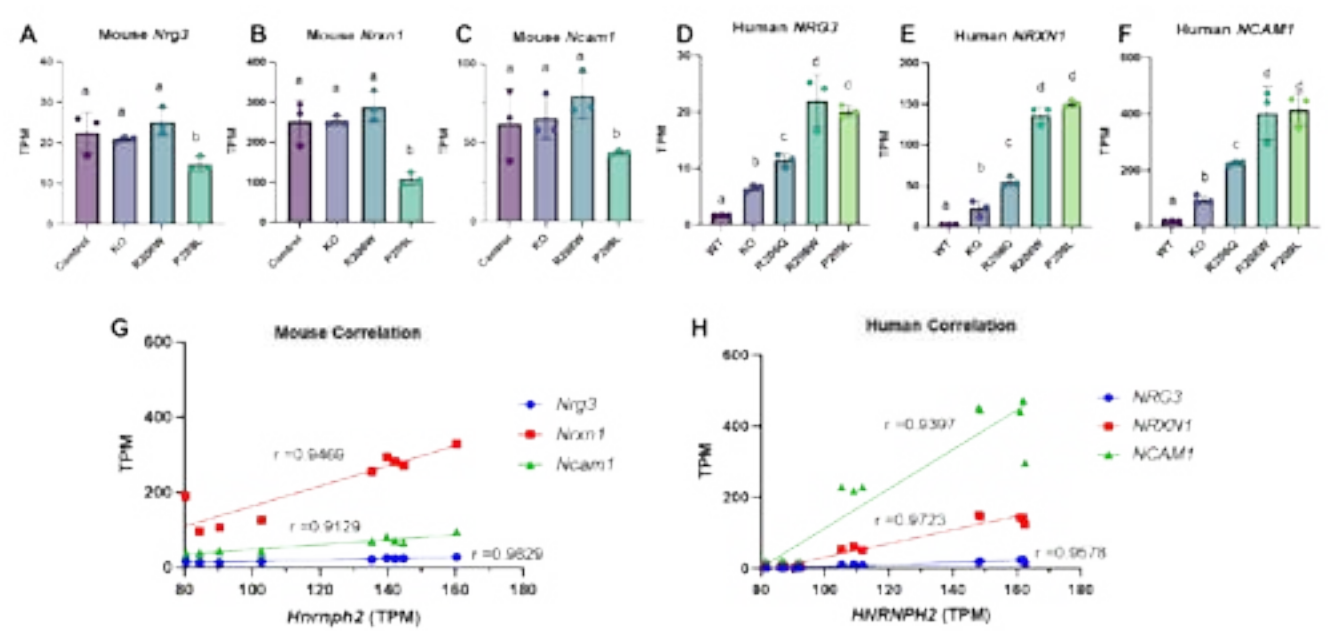

Figure 6: Bulk RNA-seq analysis for mouse cortex (A-C) and human iPSC-derived neurons (D - F) with KO or mutations at R206Q, R206W, or P209L (n = 3 biological replicates per group. *: p < 0.0001, different letters indicate statistical significance). Pearson's correlation was performed for mouse (G) and human data (H) (r: Pearson's correlation coefficient, p < 0.001).

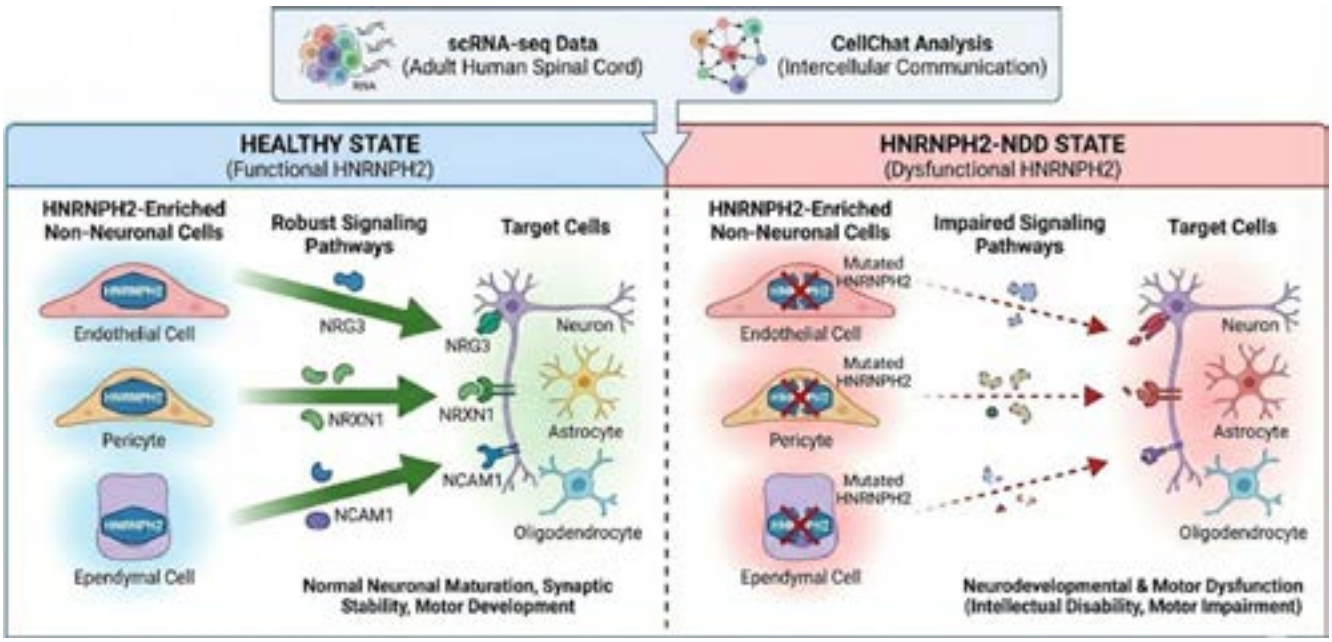

Figure 7: In this study, scRNA-seq and CellChat analysis using human spinal cord dataset revealed a potential mechanism responsible for motor function impairment caused by HNRNPH2 mutations through cell-cell communications between non-CNS cells and CNS cells. Strong NRG3, NRXN1, and NCAM1 signal from endothelial cells, pericytes, and ependymal cells to neurons, astrocytes, and oligodendrocytes, discovered in healthy spinal cords suggest that HNRNPH2 mutations in the disease may lead to disruption of the key signals, playing essential roles in neural and motor function development and maintenance.

HNRNPH2 plays a functional role in maintaining or modulating these communication networks. Disruption of HNRNPH2 could therefore impair key developmental signaling pathways, contributing to the broad neurological and motor phenotypes characteristic of HNRNPH2-RNDD (Figure 7).

Previous studies investigating the molecular mechanisms underlying neurodevelopmental disorders associated with HNRNPH2 mutations have relied mainly on bulk RNA sequencing or on genetically modified animal models [4,10,32]. Previous bulk RNA-seq and a murine model of HNRNPH2 suggested that HNRNPH2 mutations lead to impaired nuclear transport, cytoplasmic accumulation, altered RNA splicing, and inhibition of genetic compensation [4,10,32]. However, a gene expression profile from bulk RNA-seq represents the average of thousands or millions of diverse cells in the dissected tissue sample and is unable to identify cell-type-specific transcriptional signatures that define the function of distinct neuronal and glial populations. As related, the previous findings on the potential mechanism underlying neurodevelopmental disorders caused by HNRNPH2 mutations are focused on general cellular functions, rather than cell-type-specific dysfunction. Thus, our data may represent a novel finding on the cell types and signaling pathways specific to HNRNPH2-RNDD. Moreover, our findings, for the first time, identified the potential roles of non-CNS cells in the spinal cord, such as endothelial cells, pericytes, and ependymal cells, in the cell-cell communication associated with HNRNPH2-RNDD. Given our limited knowledge of the functions of these cells in neurodevelopment, our study may lead to follow-up studies to investigate biology of these cells for the development and function of the spinal cords.

Despite the novel findings, this study has several limitations. All data analyzed in this study were derived from healthy adult human spinal cord samples, and no spinal cord scRNA-seq datasets are currently available from pediatric tissue or from individuals with HNRNPH2 mutations. Because HNRNPH2-RNDD symptoms emerge in infancy and involve developmental processes, adult tissue data may not fully reflect disease-relevant signaling states. Additionally, the absence of patient-derived scRNA-seq datasets significantly limits the ability to determine how these communication pathways are altered in affected individuals. To address the limitations, we performed a comparative analysis using previously established bulk RNA-seq data of mouse cortex and human iPSCs with inserted mutations of HNRNPH2, which likely confirm the link between HNRNPH2 mutations and expressions of NRG3, NRXN1, and NCAM1. These validation data with the available disease models strongly suggests potential therapeutic targets for HNRNPH2-RNDD.

However, bulk RNA-seq cannot validate cell-cell communication identified in the scRNA-seq analysis, resulting in another limitation of this study, the paucity of in vitro validation for the identified cell-cell communication signaling in disease models. As the cell-cell signaling interactions likely occur as a part of

the systemic communication network in vivo, there is no in vitro model applicable to replicate complex in vivo systemic factors. Emerging technologies such as brain cortical organoids, organ-on-a-chip (OoC) and other types of microphysiological systems (MPS) may have the potential to develop an in vitro human model successfully replicating the complex in vivo cell-cell interactions from human iPSCs with HNRNPH2-RNDD [33-35]. However, the existing OoC and MPS are at an early developmental stage, with limited capacity. To address these limitations, future research will focus on comparing these healthy adult datasets with developing human spinal cord scRNA-seq and patient-derived data, when they become available, to determine whether the patterns identified here are conserved during development or altered in the disease state. This study is also limited by sole analysis of spinal cord, not brain. While motor planning and ambulatory symptoms in HNRNPH2-RNDD patients may result in spinal cord differences between neurotypical patients, investigating cellular differences via scRNA-seq in brain regions should enhance our understanding of cellular communication networks necessary for both development and homeostasis.

Conclusion

Our integrative scRNA-seq and CellChat analyses provide a strong foundation for understanding the biological roles of HNRNPH2 and how HNRNPH2 mutations may lead to disrupted cell-cell communication, potentially explaining the diverse motor, sensory, and developmental impairments observed in individuals with HNRNPH2-RNDD.

Author Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

All data supporting the findings of this study are available on paper. The scRNA-seq dataset of human spinal cords used in this study are available at CELLxGENE atlas.

Competing interests

The author has no competing interests to disclose.

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References

1. Bain J, Thornburg O, Salazar R, LaMarca NH, Chung W (2019) A expanded cohort study of the X-linked HNRNPH2-related neurodevelopmental disorder (P1.6-039). Neurology 92: P1.6-P039.
2. Bain JM, Cho MT, Telegrafi A, Wilson A, Brooks S, et al. (2016) Variants in HNRNPH2 on the X Chromosome Are Associated with a Neurodevelopmental Disorder in females. Am J Hum Genet 99: 728-734.
3. Bain JM, Thornburg O, Pan C, Rome-Martin D, Boyle L, et al. (2021) Detailed clinical and psychological phenotype of the x-linked HNRNPH2-related neurodevelopmental disorder. Neurol Genet 7: e551.
4. Gillentine MA, Wang T, Hoekzema K, Rosenfeld J, Liu P, et al. (2021) Rare deleterious mutations of HNRNP genes result in shared neurodevelopmental disorders. Genome Med 13: 63.
5. Grammatikakis I, Zhang P, Panda AC, Kim J, Maudsley S, et al. (2016) Alternative splicing of neuronal differentiation factor TRF2 regulated by HNRNPH1/H2. Cell Rep 15: 926-934.
6. Geuens T, Bouhy D, Timmerman V (2016) The hnRNP family: Insights into their role in health and disease. Hum Genet 135: 851-867.
7. Jepsen WM, Ramsey K, Szelinger S, Llaci L, Balak C, et al. (2019) Two additional males with X-linked, syndromic mental retardation carry de novo mutations in HNRNPH2. Clin Genet 96: 183-185.
8. Davis TJ, Salazar R, Beenders S, Boehme A, LaMarca NM, et al. (2023) A prospective, longitudinal study of caregiver-reported adaptive skills and function of individuals with HNRNPH2-related neurodevelopmental disorder. Adv Neurodev Disord 8: 445-456.
9. Vavassori S, Russell S, Scotti C, Benvenuti S (2024) Unlocking the full potential of rare disease drug development: Ethe not-for-profit sector's contributions to drug development and access. Front Pharmacol 15.
10. Korff A, Yang X, O'Donovan K, Gonzalez A, Teubner BJ, et al. (2023) A murine model of hnRNPH2-related neurodevelopmental disorder reveals a mechanism for genetic compensation by Hnrnph1. J Clin Invest 133: e160309.
11. Somashekar PH, Narayanan DL, Jagadeesh S, Suresh B, Vaishnavi RD, et al. (2020) Bain type of X-linked syndromic mental retardation in a male with a pathogenic variant in HNRNPH2. Am J Med Genet A 182: 183-188.
12. Zhu H, Bowling R, Cabrera R, Banks C, Finnell R (2024) P147: Precision medicine approaches to treatment for HNRNPH2 mutations*. Genetics in Medicine Open 2: 101044.
13. Chen G, Rodriguez-Lopez A, Wangsa D, Lomash RM, Huang X, et al. (2025) Reactivation of human X-linked gene and stable X-chromosome inactivation observed in generation and differentiation of iPSCs from a female patient with HNRNPH2 mutation. Cells 14: 1486.
14. Blum JA, Klemm S, Shadrach JL, Guttenplan KA, Nakayama L, et al. (2021) Single-cell transcriptomic analysis of the adult mouse spinal cord reveals molecular diversity of autonomic and skeletal motor neurons. Nat Neurosci 24: 572-583.
15. Chen G, Ning B, Shi T (2019) Single-cell RNA-Seq technologies and related computational data analysis. Front Genet 10: 317.

16. Islam S, Kjällquist U, Moliner A, Zajac P, Fan JB, et al. (2011) Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq. Genome Res 21: 1160-1167.

17. Jovic D, Liang X, Zeng H, Lin L, Xu F, et al. (2022) Single-cell RNA sequencing technologies and applications: A brief overview. Clin Transl Med 12: e694.

18. Verma PK, Lekkala S, Usman B, Lankireddy SV, Khadgi A, et al. (2025) Single-cell RNA sequencing methodology, analysis, applications, and future directions with special focus on cotton. Plant Biol 28: 31-44.

19. Ofengeim D, Giagtzoglou N, Huh D, Zou C, Yuan J (2017) Single-cell RNA sequencing: Unraveling the brain one cell at a time. Trends Mol Med 23: 563-576.

20. Chen Z, Shi J, Li L (2025) Application of single-cell sequencing technology and its clinical implications in parkinson's disease and alzheimer's disease: A narrative review. Advanced Technology in Neuroscience 2: 9-15.

21. Jung N, Kim TK (2023) Spatial transcriptomics in neuroscience. Exp Mol Med 55: 2105-2115.

22. Hayakawa Y, Ozaki H (2025) A practical guide for single-cell transcriptome data analysis in neuroscience. Neurosci Res 214: 9-15.

23. Jin S, Guerrero-Juarez CF, Zhang L, Chang I, Ramos R, et al. (2021) Inference and analysis of cell-cell communication using CellChat. Nat Commun 12: 1088.

24. Jin S, Plikus MV, Nie Q (2025) CellChat for systematic analysis of cell-cell communication from single-cell transcriptomics. Nat Protoc 20: 180-219.

25. Siletti K, Hodge R, Albiach AM, Lee KW, Ding SL, et al. (2023) Transcriptomic diversity of cell types across the adult human brain. Science 382: eadd7046.

26. Siletti K, Hodge R, Albiach AM, Lee KW, Ding SL, et al. CELLxGENE dataset, dissection: Spinal cord. The Human Brain Cell Atlas.

27. Seeker LA, Bestard-Cuche N, Jäkel S, Kazakou NL, Bøstrand SMK, et al. (2023) Brain matters: Unveiling the distinct contributions of region, age, and sex to glia diversity and CNS function. Acta Neuropathol Commun 11: 84.

28. Jiang A, Snell RG, Lehnert K (2024) ICARUS v3, a massively scalable web server for single-cell RNA-seq analysis of millions of cells. Bioinformatics 40: btae167.

29. Mei L, Nave KA (2014) Neuregulin-ERBB signaling in the nervous system and neuropsychiatric diseases. Neuron 83: 27-49.

30. Zhang S, Xia YY, Lim HC, Tang FR, Feng ZW (2010) NCAM-mediated locomotor recovery from spinal cord contusion injury involves neuroprotection, axon regeneration, and synaptogenesis. Neurochem Int 56: 919-929.

31. Erickson RP (2016) The importance of de novo mutations for pediatric neurological disease--It is not all in utero or birth trauma. Mutat Res Rev Mutat Res 767: 42-58.

32. Kelvington BA, Abel T (2023) hnRNPH2 gain-of-function mutations reveal therapeutic strategies and a role for RNA granules in neurodevelopmental disorders. J Clin Invest 133: e171499.

33. Lall D, Workman MJ, Sances S, Ondatje BN, Bell S, et al. (2025) An organ-chip model of sporadic ALS using iPSC-derived spinal cord motor neurons and an integrated blood-brain-like barrier. Cell Stem Cell 32: 1139-53.e7.

34. Sances S, Ho R, Vatine G, West D, Laperle A, et al. (2018) Human iPSC-derived endothelial cells and microengineered organ-chip enhance neuronal development. Stem Cell Reports 10: 1222-1236.

35. Leung CM, de Haan P, Ronaldson-Bouchard K, Kim GA, Ko J, et al. (2022) A guide to the organ-on-a-chip. Nature Reviews Methods Primers 2: 33.