Damaging Variants in *HNRNPUL2* Result in a Novel HNRNP-Related Neurodevelopmental Disorder

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INTRODUCTION

The *HNRNP* genes, encoding for heterogeneous nuclear ribonuclear proteins, have been shown to have significant enrichment of damaging *de novo* variation among neurodevelopmental disorder cohorts. To date, there are nine *HNRNP* genes with well described neurodevelopmental disorder associations, collectively termed the HNRNP-Related Neurodevelopmental Disorders (RNDDs), and multiple candidate genes. Here, we have found evidence that damaging variants in *HNRNPUL2*, encoding heterogenous nuclear ribonucleoprotein U-like 2, contribute to HNRNP-RNDDs. Multiple studies have shown that damaging variants in *HNRNPUL2* are significantly enriched among autism and NDD cohorts.

We describe HNRNPUL2-RNDD, consisting of global developmental delay, normal cognition to moderate intellectual disability, behavioral differences, growth delay, seizures, and incoordination. Highlighted by the number of cases identified in the SPARK cohort, autism spectrum disorder is observed in over half of individuals identified, and HNRNPUL2-RNDD has the highest autism prevalence among the HNRNP-RNDDs. Additional natural history study data is being collected utilizing the Geneial platform.

RNAseq on fibroblasts derived from one individual with a nonsense variant (p.Tyr214*) showed a significant reduction in *HNRNPUL2* gene expression. Top dysregulated genes were enriched in pathways relevant to neuronal development, Wnt signaling, and parathyroid hormone response. Future RNAseq from induced neurons will likely show additional neurodevelopmental genes that are dysregulated.

Furthermore, a *Drosophila* model has been developed using a patient's missense variant that shows loss-of-function (p.Glu707Ala).

This international endeavor expands the HNRNP-RNDDs phenotypically and molecularly.

METHODS

- Utilizing connections through the HNRNP Family Foundation, SPARK and Simons Searchlight cohorts, GeneMatcher, Baylor Genetics, and the literature and patient databases, we have identified 28 individuals with de novo and inherited loss-of-function variants in HNRNPUL2. Clinic notes for these individuals were reviewed.
- Skin biopsies were collected at HNRNP Family Foundation annual meetings by clinicians and generated into fibroblasts by the Columbia University Stem Cell Core.
- RNAseq was performed on patient fibroblasts. RNAseq datasets were processed using NF-core RNAseq version 3.13.2 [https://doi.org/10.5281/zenodo.1400710]. Reads were trimmed using trimmomatic (cutadapt v3.4) and mapped using STAR (v2.7.9a). Mapped reads were quantified using Salmon (v1.10.1). All analyses were performed on the Triton Shared Computing Cluster TSCC [[https://doi.org/10.57873/T34W2R]].

Abbreviations: ADHD (attention deficit hyperactivity disorder), Autism Spectrum Disorder (ASD), DD (developmental delay), ID (intellectual disability), learning disability (LD), HNRNP (heterogeneous nuclear ribonuclear protein), RNAseq (RNA sequencing), RNDD (related neurodevelopmental disorder), Sensory Processing Disorder (SPD)

Mutational Spectrum

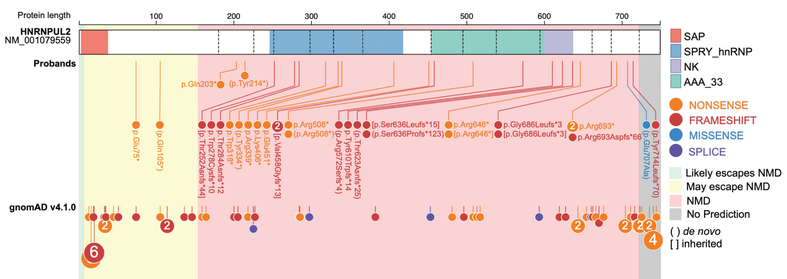

Figure 1. Mutational landscape of *HNRNPUL2* variation. Probands' variants and variants predicted to result in loss-of-function from gnomAD v4.1.0 are shown (accessed Sept 2025). 60% of variants with known inheritance occurred *de novo* (in parentheses) while 40% were inherited (in brackets). At least one inherited variant was from an affected parent with febrile seizures, learning difficulties, and ADHD. Loss-of-function variants present in gnomAD and inherited from parents supports a mild neurodevelopmental phenotypes in some individuals and/or incomplete penetrance. NMDetective predictions for nonsense mediated decay (NMD) are indicated by green (score = 0.12, likely escapes NMD), yellow (score = 0.41, may escape NMD), red (score = 0.65, NMD), and black (no prediction).

Phenotypic Spectrum

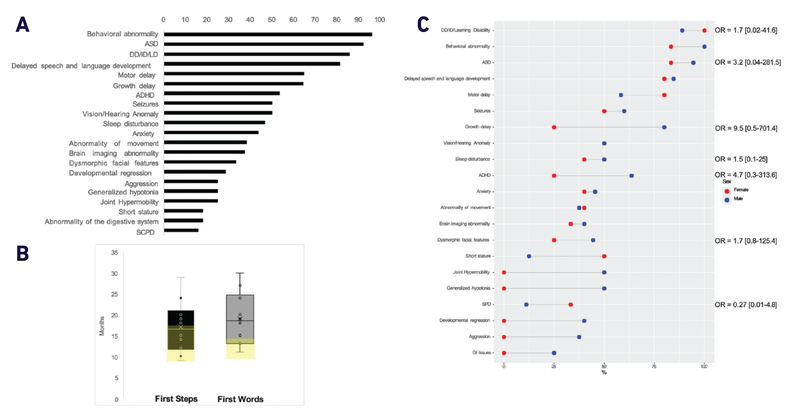

Figure 2. Phenotypic spectrum of *HNRNPUL2* variation. (A) Phenotypes across all HNRNPUL2-RNDD probands. Behavioral differences, in particular ASD, are prevalent in almost all individuals with damaging *HNRNPUL2* variants. Cognitive skills range from moderate DD/ID/LDs to normal. (B) Milestones of HNRNPUL2-RNDD probands. Of those with available information, speech delay was more prominent than motor delay. Yellow indicates normal milestones range. (C) Phenotypic differences between males (n = 20) and females (n = 8). Growth delay/failure to thrive was far more common among males, although short stature was more common among females. ADHD, ASD, and sleep disturbances were more likely to be present in male probands, while SPD was diagnosed more among females. Males were more likely to have subtle dysmorphic facial features and cognitive delays. Notably, HNRNPUL2-RNDD has the highest ASD prevalence of all the reported HNRNP-RNDDs. Remote data collection is ongoing using the Geneial platform.

Functional Studies

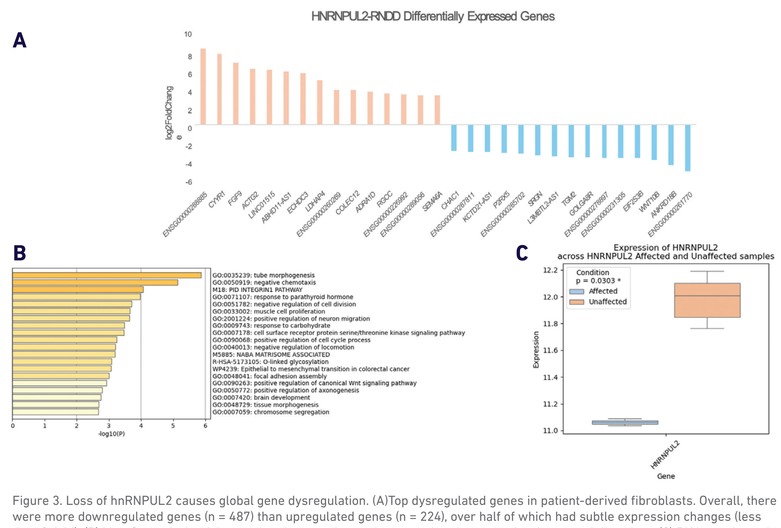

Figure 3. Loss of hnRNPUL2 causes global gene dysregulation. (A)Top dysregulated genes in patient-derived fibroblasts. Overall, there were more downregulated genes (n = 487) than upregulated genes (n = 224), over half of which had subtle expression changes (less than 2-fold). (B) MetaGo analysis of dysregulated genes shows changes related to neurodevelopmental disorders. (C) RNAseq showed significant decreased expression of *HNRNPUL2* in patient-derived fibroblasts (n = 1), consistent with haploinsufficiency.

CONCLUSIONS

- This work supports *HNRNPUL2* as a candidate gene for autism spectrum disorder and behavioral differences broadly.
- Loss-of-function of hnRNPUL2 appears to be the pathomechanism. Protein studies are required to assess for true loss-of-function of hnRNPUL2.
- Even in fibroblasts, neurodevelopmental relevant genes and pathways are shown to be disrupted. These include genes already associated with ASD, such as *CDKL5* and *PHIP*, as well as genes known to be involved in axonal guidance and neuronal migration.
- Patient-derived fibroblast-transdifferentiated neurons from our patient are actively undergoing RNAseq. Additional samples and modeling will be necessary to fully understand the molecular impact of HNRNPUL2-RNDD.
- Overall, our data support that HNRNPUL2-RNDD should be included among the HNRNP-RNDDs.

Check out the HNRNPUL2-RNDD (and other HNRNP-RNDDs!) at Melanie Mew's poster (Poster 7048, presented on Thursday!)

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