Pathogenic CLP1 p.R140H mutation alters mRNA processing in human motor neurons

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Geneva LaForce[1], Jordan Farr[1], Evren Gumus[2], Cydni Akesson[1], Merve Yazol[3], Eric J Wagner[4], Polyxeni Philippidou[5], Ashleigh E. Schaffer[1]

1. Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH
2. Department of Medical Genetics, Faculty of Medicine, University of Harran, Sanliurfa, Turkey
3. Department of Radiology, Sanliurfa Education and Research Hospital, Sanliurfa, Turkey
4. Department of Biochemistry and Molecular Biology, University of Texas Medical Branch at Galveston, Galveston, TX
5. Department of Neurosciences, Case Western Reserve University, Cleveland, OH

Pontocerebellar Hypoplasia Type 10 (PCH10) is a pediatric neurodegenerative disorder caused by homozygous p.R140H mutations in CLP1 that lead to hypoplasia of the cerebellum and brainstem with motor neuron degeneration. CLP1 is a multifunctional RNA kinase implicated in tRNA splicing and mRNA 3'-end formation. Prior studies proposed tRNA biogenesis defects may be disease causing; however, the pathogenic mechanism remains unknown. Here, we show motor neuron disease in PCH10 is likely caused by aberrant mRNA processing. We describe PCH10 patients with penetrant motor neuron and variable brain features, which was phenocopied in mice. To define RNA biogenesis defects in PCH10, we generated iPSC-derived motor neurons from PCH10 patients and an unaffected relative. During differentiation, we found reduced cell density in PCH10 compared to control motor neurons, possibly akin to motor neuron degeneration. Genome-wide transcriptome analysis of mRNA and tRNA processing and abundance in PCH10 motor neurons found global dysregulation of mRNA expression, splicing, and polyadenylation, without changes in tRNA expression or fragment accumulation. We identified a 3'-end bias in mRNA splice donor and acceptor inclusion in PCH10 motor neurons, particularly within 3' UTRs. Further, PCH10 motor neurons showed increased usage of distal poly(A) sites with a corresponding decrease in intronic polyadenylation. These findings were associated with a positive correlation between gene length and expression. Taken together, these results suggest CLP1 may function late in transcription to control the ratio of full length and alternative isoforms expressed. The genes impacted by CLP1 mutation are found within pathways critical for neuronal function, including ion homeostasis and calcium signaling. A subset of differential genes and events seen in PCH10 motor neurons overlap with ALS, hinting they may be drivers of disease or biomarkers of neurodegeneration. Our data expand

the clinical spectrum of PCH10, develop accurate pre-clinical models for the disease, and link CLP1 p.R140H mutation with dysregulation of mRNA processing in motor neuron degeneration.