Circulating His-tRNA Synthetase is Reduced in Patients Harboring the Usher Syndrome Type 3B-linked mutation Y454S

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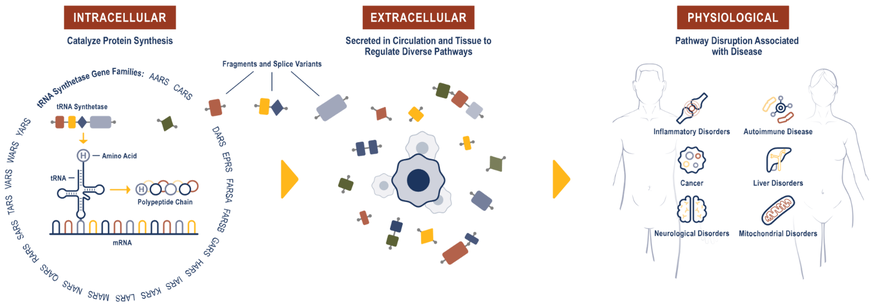
Abstract

The His-tRNA synthetase (HARS) WHEP-domain can be liberated from the full-length enzyme by both alternative splicing and proteolysis to signal extracellularly. This signaling can be important in regulating physiology, which is exemplified by its association with anti-Jo-1+ disease wherein neutralization of extracellular HARS by high-titer antibodies leads to an aberrant tissue-immune response characterized by interstitial lung disease and myositis.

While there have been no human disease linkages for mutations occurring in the HARS WHEP-domain, a mutation outside the catalytic domain of HARS, Y454S, is associated with the development of Usher Syndrome Type 3B (USH3B) in young children. Biochemical characterization of this mutation revealed minimal enzymatic effects; however, the mutant enzyme exhibited worsened thermal stability. We hypothesized that the inherent instability introduced by the Y454S mutation could result in decreased levels of extracellular, circulating HARS protein available for signaling. Utilizing plasma samples from a recent clinical trial involving children with the Y454S homozygous mutation, circulating HARS was assessed and found to exhibit strikingly lowered circulating HARS levels compared to age-matched healthy controls. These findings bolster previous protein stability results and may suggest a functional role for extracellular HARS that is diminished in individuals with Y454S mutations, perhaps providing rationale for some of the overlapping clinical features observed between anti-Jo-1+ disease and USH3B.

Background



The study of various tRNA fragments and splice variants has uncovered novel functions of tRNA synthetases that signal extracellularly to modulate physiological functions. HARS is found in human circulation and its activity modulates the trafficking of immune cells to sites of inflammation in pre-clinical mouse models. This suggests a role for HARS and its WHEP-domain in dampening the immune response and restoring homeostasis in the setting of chronic inflammation. A deficit in this extracellular signaling could be a source of disease symptoms experienced by USH3B children.

Materials and Methods

Biochemical Characterization

HARS domain structures diagram. The Y454S mutation is located in the anticodon binding domain.

Aminoacylation Activity. Aminoacylation reactions were performed at 25 °C in 50 mM HEPES buffer (pH 7.5), containing 300 µM L-His, 6.6 µM [^{3}H]-L-His, 500 µM of yeast tRNA, 4 µg/mL pyrophosphatase, 10 mM $MgCl_2$, 4 mM ATP, 2 mM DTT and HARS WT or Y454S protein that varied in concentration from 25-150 nM. Aliquots were removed every 30 sec, and quenched on filter papers (Whatman, 3 mm) equilibrated with 5% trichloroacetic acid (TCA). The filters were washed three times with 5% ice-cold TCA and twice with 80% ethanol. Radioactive counts in the precipitates were quantitated by scintillation counting. Initial velocities for each reaction were fitted by linear regression and plotted against enzyme concentrations.

Melting Curves. Melting curves were measured on a ViiA7 instrument using the Protein Thermal Shift Dye Kit from ThermoFisher. Dye stock was prepared by adding 2 µL dye to 40 µL ddH_2O. HARS WT and Y454S proteins were diluted to 0.5 mg/mL in 1x PBS, 1 mM DTT (pH 7.4) with or without 34 mM L-His. 100 µL of each protein sample was mixed with 5 µL of diluted dye stock and 20 µL/well of each mixture was transferred to a 384 well plate. Each sample was run in 4 wells for technical replicates.

Circulating HARS levels

Patient plasma samples. Heparin plasma tubes were used to collect blood samples from control individuals and USH3B patients. Following centrifugation plasma samples were stored frozen until analysis.

HARS immunoassay. The HARS immunoassay utilized the MSD (Meso Scale Diagnostics) platform with a capture antibody specific for the HARS WHEP domain (❶) and a biotinylated detection antibody which recognizes the HARS aminoacylation domain (❷). HARS antibodies were produced at AbCellera Biologics, Inc. (Vancouver, Canada) and Scripps Research (San Diego, CA) and the recombinant protein standard was produced at Syngene (Cambridge, UK). Streptavidin-SULFO tag (❸) (MSD Rockville, MD) was used as a secondary detection and plates were read on a MESO quickplex SQ120MM (MSD).

Population comparison. USH3B children had plasma collected throughout a 2-year clinical study with 3-6 plasma samples per patient being analyzed based on availability. Sample concentrations were averaged for each individual patient to balance the population mean.

References

Abbott JA, Guth E, Kim C, Regan C, Siu VM, Rupar CA, Demeler B, Francklyn CS, Robey-Bond SM. The Usher Syndrome Type IIIB Histidyl-tRNA Synthetase Mutation Confers Temperature Sensitivity. Biochemistry. 2017 Jul 18;56(28):3619-3631. doi: 10.1021/acs.biochem.7b00114. Epub 2017 Jul 7. PMID: 28632987; PMCID: PMC9677509.

Results

Consistent with published data (Abbott, 2017) Y454S mutation reduces protein stability

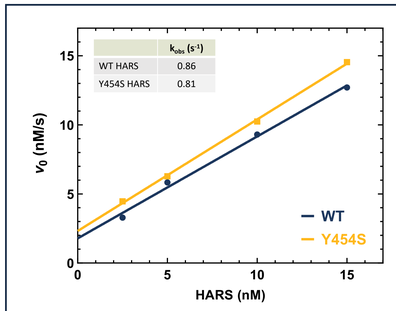


Figure 1: Aminoacylation activity of HARS and Y454S HARS. Initial velocities of amino-acylation at different HARS concentrations are plotted. WT and Y454S HARS have observed catalytic turnover rates of 0.86 s^{-1} and 0.81 s^{-1} respectively, indicating the enzymatic activity of WT and Y454S HARS is similar.

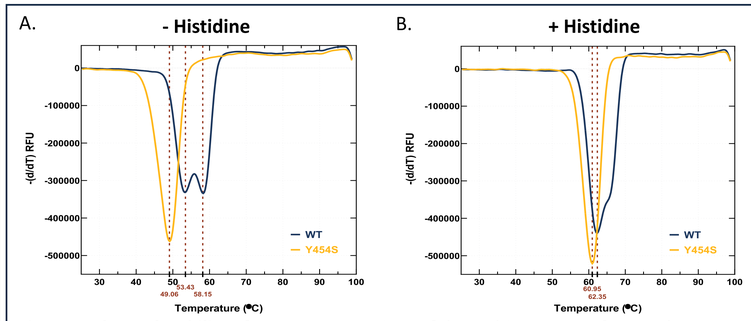


Figure 2: Thermal stability of HARS. A comparison of the melting curves of WT and Y454S HARS. (A) In the absence of histidine, the melting curve for the Y454S mutation shows a left shift compared to WT HARS with WT HARS having a maximum stability at temperatures 63.43-68.16˚C and Y454S at 48.06˚C. This indicates the Y454S protein is less stable at higher temperatures than WT HARS. (B) The presence of histidine stabilizes both the WT and Y454S, with a minor decrease in thermal stability observed in the Y454S HARS compared to WT.

USH3B children (Y454S homozygous) have decreased extracellular HARS

Circulating HARS by population		
	Control	USH3B
N (# patients)	20	14
range max (pg/mL)	5855	1778
range min (pg/mL)	654	225
mean (pg/mL)	1983	920

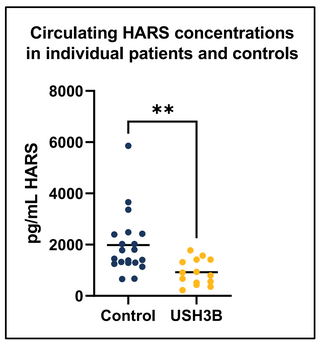
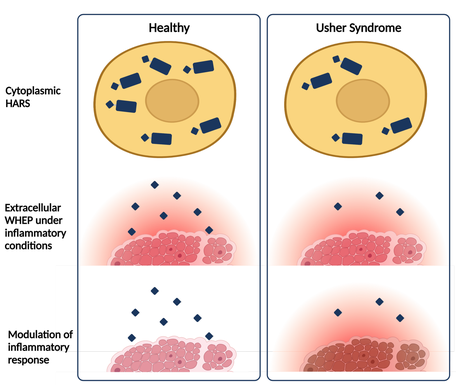


Figure 3: Circulating HARS levels of age-matched healthy control individuals and USH3B (Y454S homozygous) patients. Plasma was collected from 20 age-matched control individuals and 14 homozygous Y454S children (ages 0-18 years). (Left) Control plasma levels ranged from 654-5855 pg/mL while USH3B children had 225-1778 pg/mL. The population average for control HARS levels was 1983 pg/mL and USH3B population average HARS levels was 920 pg/mL. (Right) While both populations exhibit a wide range of circulating HARS level, there is a statistically significant ($p<0.005$) decrease in circulating HARS levels between populations when using an unpaired t-test (Prism 9.3.1).

Discussion and Conclusions

The observed shift in thermal stability in the Y454S mutation and minimal changes in enzymatic activity hints that symptoms in Usher Syndrome patients may be due to decreases in available HARS protein, and not the canonical functionality of HARS as a synthetase. Furthermore, the thermal instability of HARS in the absence of histidine would be more pronounced in HARS protein found extracellularly since physiological concentrations of circulating histidine is not enough to stabilize the shift in melting curve observed in the Y454S mutation. The thermal instability of HARS containing the Y454S mutation could mean that less extracellular HARS is present in Usher Syndrome patients who are homozygous for the Y454S mutation.



We compared circulating HARS levels in 14 Usher Syndrome children to age matched controls and found that Usher Syndrome patients indeed had overall lower levels of HARS present in plasma. This observation along with the known decrease in thermal stability of Y454S mutation would indicate that USH3B patients could experience even greater depressions in extracellular HARS during periods of fever. The increase in symptom severity experienced by USH3B patients during periods of fever could be connected to a decrease in extracellular HARS. We also know the severe symptoms observed in anti-Jo-1+ disease patients is caused by antibodies that are neutralizing the ability of extracellular HARS to modulate immune response, exemplifying the importance of the HARS WHEP domain in disease progression. This exemplifies the importance of extracellular signaling of HARS and could explain the disease progression observed in USH3B patients.

Decreased extracellular HARS may cause patients with Usher Syndrome to experience decreased immunomodulation through the WHEP domain of HARS, which could lead to worsened symptoms during periods of inflammation.