Novel ALDH18A1 mutation

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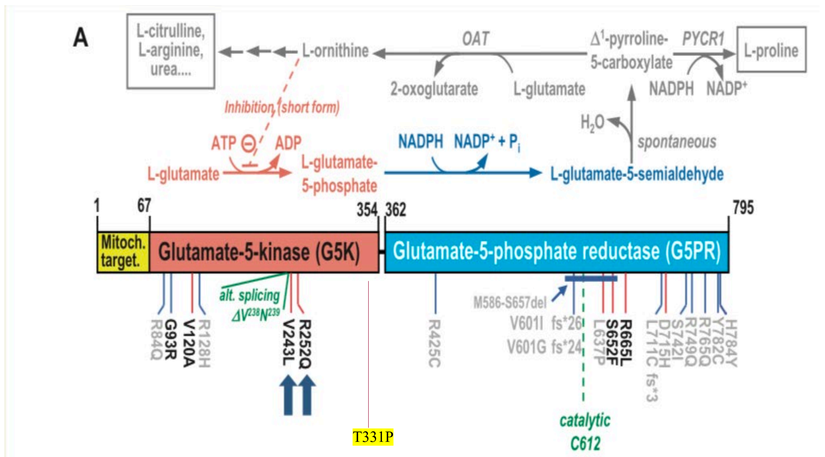
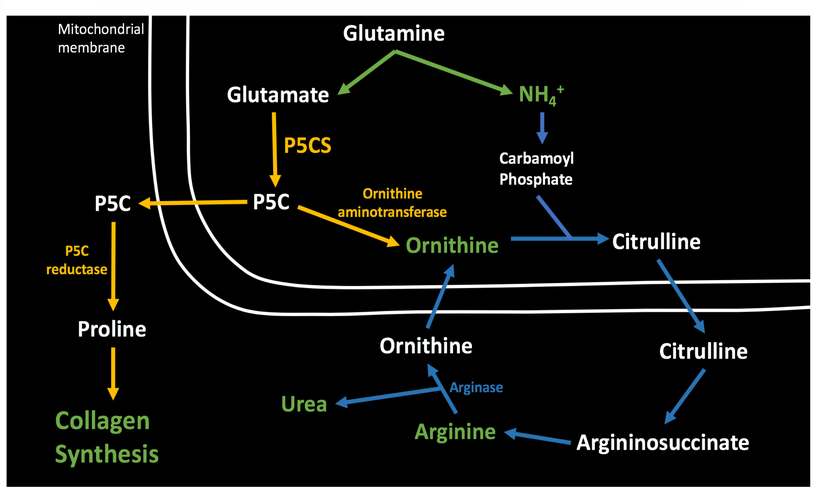
Abstract

Mutations in the gene ALDH18A1 have been identified in patients with neurocutaneous disorders. The gene ALDH18A1 encodes the enzyme P5CS, which facilitates the first step in the de novo synthesis of proline and ornithine. Proline is subsequently used in collagen synthesis (likely the source of cutaneous phenotypes), and ornithine is a key component of the urea cycle (which may explain the neurological deficits in patients). This project focuses on the functional characterization of a specific ALDH18A1 homozygous variant (T331P) found in four patients within two different, unrelated families, that presented with neurological deficiencies but no cutaneous findings. We aimed to prove the pathogenicity of this specific variant by studying its impact on the level of collagen and other metabolites, and the localization of the WT and T331P enzyme using ALDH18A1 KO HEK293 cells as a system in the absence of patient-derived fibroblasts. Our functional studies demonstrated that both WT and T331P P5CS protein are highly stable and expressed at roughly equal levels in the KO cells. Both WT and T331P enzyme localized to mitochondria, although these organelles appeared enlarged in the T331P P5CS-expressing cells. Analysis of metabolite levels in WT, ALDH18A1 KO, KO + WT ALDH18A1, and T331P ALDH18A1 was performed using nuclear magnetic resonance (NMR)-based metabolomics. These experiments showed the reduced ability of the T331P enzyme to increase proline levels, compared to the WT enzyme, when introduced into the KO background. Collectively, our observations support the likely pathogenic classification of this variant which we expect will be confirmed once experiments on patient-derived tissue can be carried out. We speculate that the T331P P5CS enzyme provides enough de novo proline synthesis to spare the patients from cutaneous phenotypes, but insufficient ornithine synthesis in neurons to avoid metabolic deficits within the brain.

Introduction

- The gene ALDH18A1 encodes the protein "Δ^1-pyrroline-5-carboxylate synthase" (P5CS).
- P5CS is the enzyme that converts Glutamate to Δ^1-pyrroline-5-carboxylate (P5C).
- P5C is a common intermediate in the de novo synthesis pathway for both Proline and Ornithine.
- Proline is a key component in the protein Collagen. Due to the structure of collagen being very reliant upon proline, we believe that a reduction in proline synthesis can result in collagen defects
- We are studying two cases that are homozygous for the mutation 991A>C (T331P) in ALDH18A1
  - These cases are very interesting because this specific mutation has not been reported elsewhere, and both patients are located in Charleston. To make it even more interesting, they are not related in any way.
- Our cases are slightly different than other cases involving ALDH18A1, because these two patients don't present with any of the cutaneous symptoms, only the neurologic symptoms.





Methods

- **Heavy Isotope Labeling:** Using NMRI, radiolabeled glutamate was administered to cells to determine the amount of proline derived from glutamate verses Proline formed de novo in the wild type verses variant cell lines.
- **Western Blot:** Using gel electrophoresis, levels of the enzyme being studied (P5CS) were measured to compare the amount synthesized in the wildtype verses variant cell lines
- **Collagen Assay:** Using an Abcam Total Collagen Assay kit, the levels of collagen formed in the wildtype verses variant cell lines were measured
- **Antibody Tracking:** Using rabbit antibody tracking with a fluorescent microscope, the location of the enzyme P5CS was tracked in the wildtype, KO, rescue, and variant cell lines.

Results

Figure 1

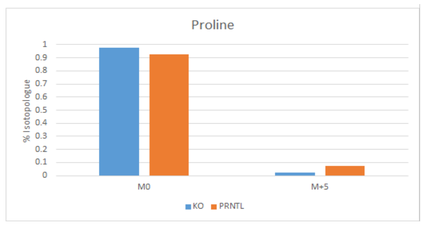


Figure 2A

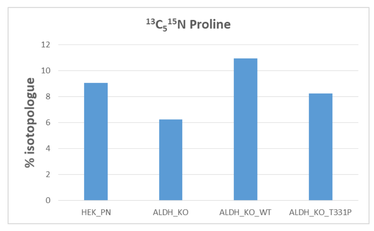


Figure 2B

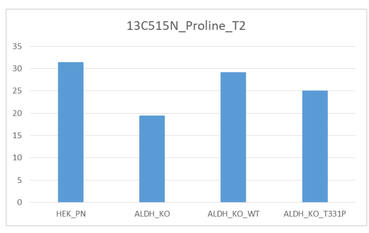
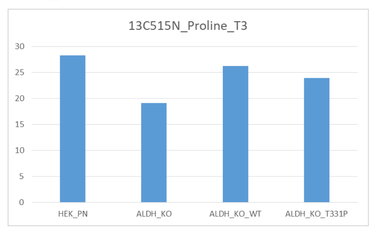


Figure 2C



- **Figure 1:** Levels of radiolabeled proline in wildtype cells compared to ALDH18A1 KO cells
- **Figure 2A-C:** Levels of radiolabeled proline in wildtype cells, knockout cells, WT rescue cells, and T331P variant cells

Figure 3A

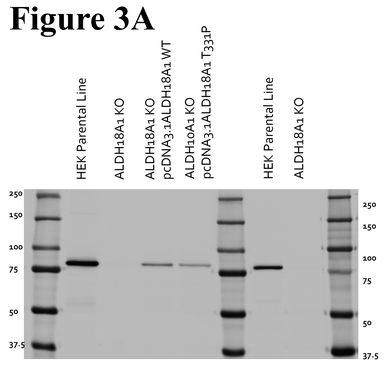


Figure 3B

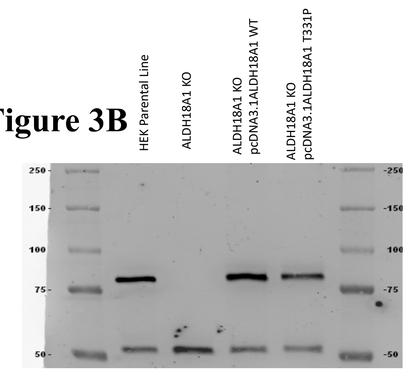


Figure 4A

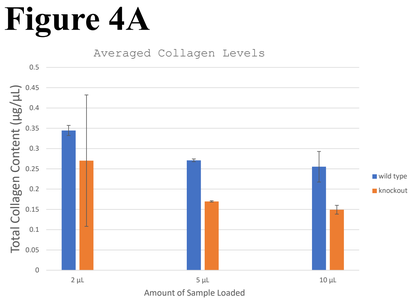


Figure 4B

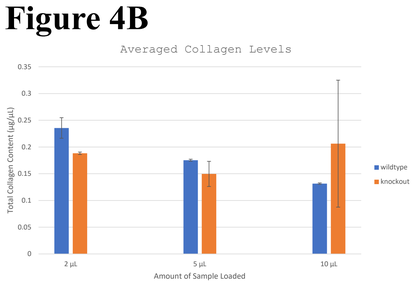


Figure 3A-B: Western blot with wildtype, ALDH18A1 KO, ALDH18A1 rescue, and T331P variant

Figure 4A-B: Two trials of the total collagen assay

Results

Figure 5

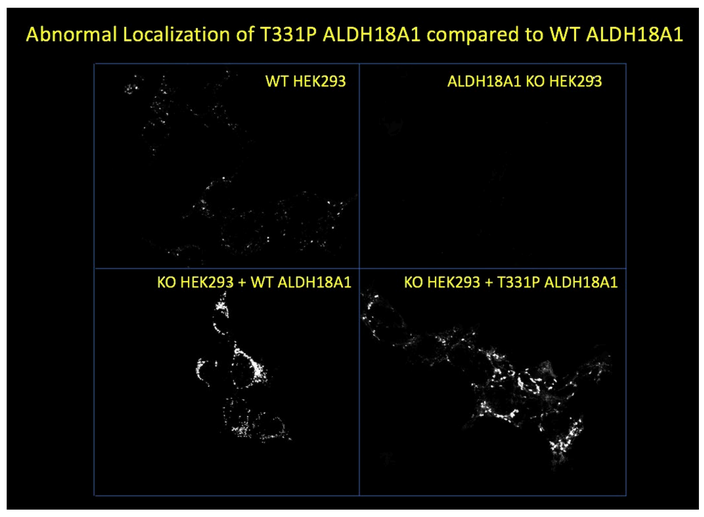


Figure 5: Antibody tracking showing the cellular location of the protein P5CS

Discussion

- The heavy isotype labeling results show that the variant T331P results in lower levels of proline formed from glutamate.
- The western blot results show that the variant T331P resulted in lower levels of the protein P5CS
- The results of the total collagen assay show that the levels of collagen are lower in the knockout cells
- The results of the antibody tracking show abnormal localization of the protein P5CS in the variant when compared to the wildtype
- For our particular cases, we are seeing neurological problems but no cutaneous symptoms.
  - This particular mutation could be causing localization problems or causing the protein to aggregate, reducing the functionality/efficiency.
  - There could be enough de novo Proline to spare matrix phenotypes, but it could be neurotoxic for some other reason
    - Proline is a known neurotransmitter, so deficiencies in Pro could lead to a bunch of issues in the CNS.
  - There could be another, currently unknown, function for P5CS that this particular mutation impacts

Future Directions

- Future experiments will include measuring levels on ornithine in the variant cells, because the enzyme P5CS impacts de novo ornithine synthesis as well.
- These experiments were done on human embryonic kidney cells in a laboratory setting. Future experiments should be conducted using actual patient tissue

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