REPORT FOR PATHOLOGICAL SOCIETY SMALL GRANT SCHEME AWARD: GRANT REFERENCE NO. 3900430

Title: Elucidating The Pathogenesis Of Mitochondrially Inherited Tubulointerstitial Kidney Disease

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Background: Chronic kidney disease (CKD) represents a significant and growing health burden. However, precise molecular mechanisms underpinning CKD pathogenesis are poorly defined and novel therapeutic agents are urgently required. Mitochondrial dysfunction can contribute to CKD, and most pathogenic mutations in the mitochondrial genome can affect protein translation. However, the role of aberrant protein translation in CKD has not been extensively explored. In this project, I study a family with isolated renal disease as a result of a mutation in the mitochondrial genome (m.547A>T). This provides an excellent opportunity to understand kidney-specific pathogenic mechanisms, and the role defective protein translation plays in CKD development.

Original Aims: Transcriptomic profiling of patient cells with m.547A>T mutation

Results

1. **Renal (m.547A>T) mutants show reduced global protein translation rate**

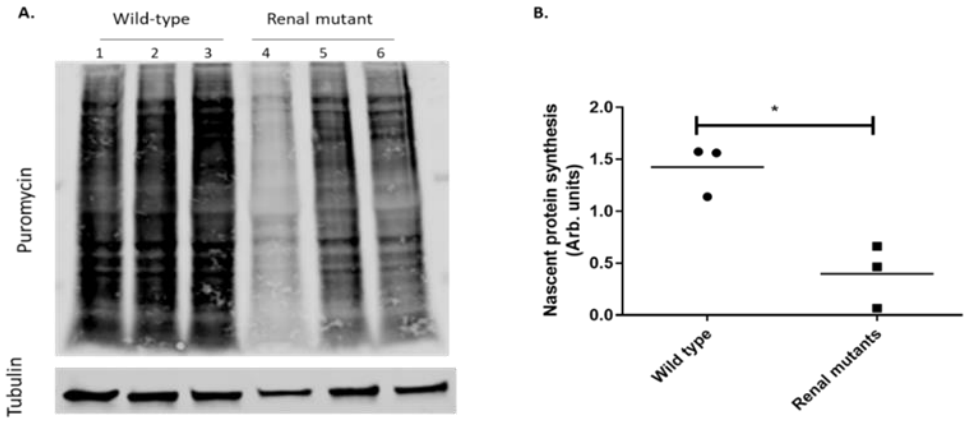

Figure 1. Assessment of protein translation rate using SUnSET assay.[1] (A) wild-type fibroblasts from 3 different individuals (lanes 1-3) and fibroblasts from 3 different m.547A>T patients (lanes 4-6) were exposed to puromycin (10µg/mL) in the culture medium for 10 minutes prior to harvesting of the total cell lysate. Western blot using mouse puromycin antiserum and rabbit anti-tubulin antibody. (B) Quantification of data in (A). * p value =0.01 (unpaired t-test).

2. **No protein translation defect identified in cells from patients from another tissue-specific disease caused by a mutation in mitochondrial genome (Leber's hereditary optic neuropathy, LHON).** This raises the possibility that the kidney is particularly susceptible to defects in protein translation.

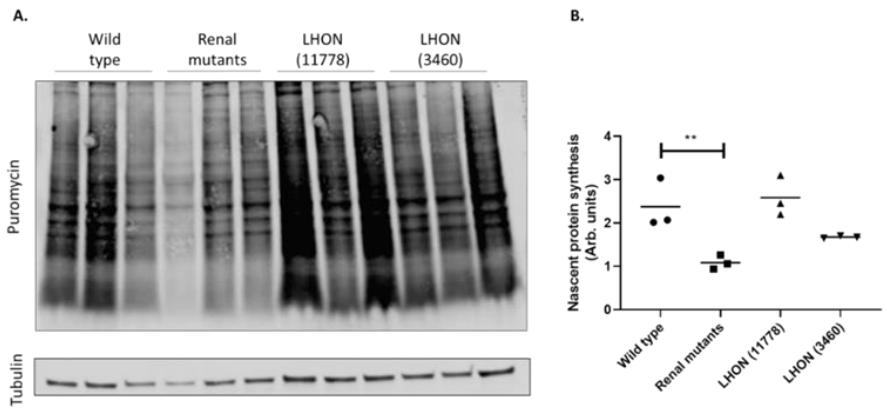

Figure 2. Assessment of protein translation in m.547A>T and LHON fibroblasts. (A) The SUnSET assay was performed in fibroblasts from the following groups (cells derived from 3 different individuals per group): wild-type volunteers, patients with the m.547A>T mutation ('Renal mutants'), LHON patients with the m.G11778A mutation [LHON (11778)] and LHON patients with the m.G3460A mutation [LHON (34600]. (B) Quantification of data in (A). One-way ANOVA followed by Dunnett's multiple comparisons test. **p=0.008

3. **Insight into potential mechanism: Sequencing of transfer/t-RNA encoded by nuclear and mitochondrial genome**. Renal mutants showed decreased expression of mitochondrially-encoded tRNA, but not tRNA encoded by nuclear genome. No differentially expressed tRNA identified in LHON mutants.

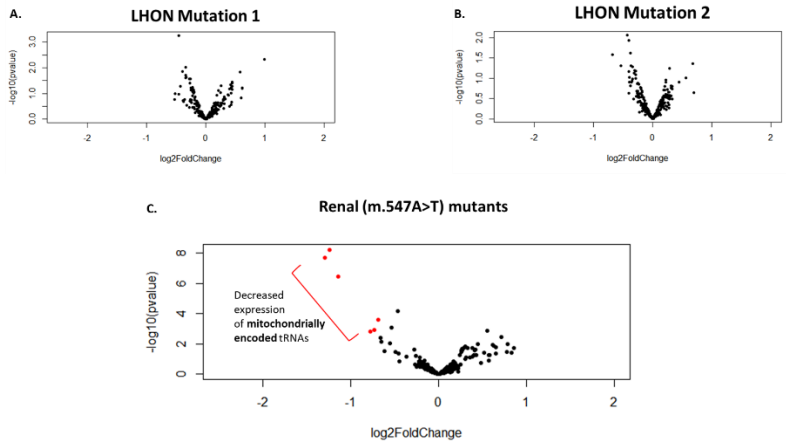

Figure 3. tRNA sequencing (tRNA-Seq) was performed to identify potential mechanism underlying protein translation defect identified in m.547A>T renal mutants. RNA analysed from 3 separate patients in each group. Volcano plots shown comparing wild-type tRNA expression (nuclear and mitochondrial genome) to: LHON mutants (A,B) and renal mutants (C).

Conclusion: Transcriptomic profiling revealed decreased expression of mitochondrially-encoded tRNA in m.547A>T renal mutants, but not in another tissue-specific disease caused by a mutation in the mitochondrial genome. This may explain the protein translation defect identified in renal mutants. Further work will focus on global RNA characterisation of m.547A>T mutants under conditions of stress and understanding the role of protein translation defects in a murine model of CKD.

Reference

1. Goodman CA, Hornberger TA. Measuring protein synthesis with SUnSET: A valid alternative to traditional techniques? *Exercise and Sport Sciences Reviews*. 2013;41(2):107-115. doi:10.1097/JES.0b013e3182798a95