A Case Study: Analysis of the Mitochondrial tRNA-Ile Gene for a Mutation Linked to Renal Magnesium Wasting

Single nucleotide point mutations within mitochondrial genes are linked to a number of maternally inherited diseases. Recently, an adult female patient was referred for clinical evaluation at Medical University of South Carolina (MUSC) and presented with hypertension, hypercholesterolemia, and hypomagnesmia. These symptoms are consistent with a known mitochondrial mutation in the isoleucine transfer RNA gene (tRNA-Ile) flanking the anticodon (bp 4293). We hypothesized that the tRNA-Ile mutation underlies the symptoms exhibited by this patient. Using restriction enzyme fragment analysis and cloning techniques, we amplified the mitochondrial tRNA-Ile gene from whole blood. Restriction digestion of this product with *Taq*1 did not produce a pattern of restriction digestion consistent with the reported mutation. Further, DNA sequence analysis revealed no mutation at tRNA-Ile 4293. A novel mutation was discovered in one of eleven tRNA-Ile PCR clones sequenced and resulted in a T>C transition at nucleotide 4327. Although further confirmation is necessary, these data suggest the possibility of a novel mutation in the mitochondrial tRNA-Ile gene of a patient with low plasma magnesium. Identification of genetic mutations will help us to focus studies of disease mechanisms.

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Introduction

Mitochondrial transfer RNAs (tRNA) play an important role in mitochondrial protein processing as well as accessory roles in nucleotide processing. Despite the major role mitochondrial tRNAs play in homeostatic function, these genes are prone to mutation. Mitochondrial tRNAs appear to evolve at a much higher rate, 100× that of their nuclear counterparts.[1] More than 250 disease-associated mutations have existed for mitochondrial genes, and even though tRNA genes constitute only 10% of the mitochondrial genome, over 100 mutations are located in the tRNA genes.[2]

Recently, an adult female patient was referred to the Medical University of South Carolina (MUSC) presenting with renal magnesium wasting resulting in hypomagnesemia, dislipidemia, hypertension, high cholesterol, diabetes and hypocalcuria. Based on low urine calcium and the absence of kidney stones, paracellin dysfunction was ruled-out. The symptoms were consistent with isolated dominant hypomagnesemia with hypocalcuria; however, this disease presents at childhood not in adulthood.[3] Alternatively, these symptoms were also consistent with those reported for individuals carrying a mutation in the mitochondrial tRNA for isoleucine at base pair (bp) 4293.[4] Individuals carrying the 4293 bp mutation characteristically exhibit hypomagnesemia, hypertension, and hypercholesterolemia.[5] Based on the parallels between individuals carrying the thymidine-to-cytidine 4293 bp tRNA-Ile mutation and this patient, we hypothesized that this patient also carried the tRNA-Ile 4293 bp mutation.

Methods

A heparinized blood sample was drawn after obtaining patient consent, following MUSC procedural guidelines.

DNA isolation and PCR

DNA was isolated from whole blood taken from the patient by heparin-EDTA syringe using Trizol® (Invitrogen, Carlsbad, Calif). Polymerase chain reaction (PCR) was used to amplify the full-length mtRNA-Ile gene from 80 ng total DNA using four primer sets designed to flank the ends of the gene starting at positions 4148 to 4355 of the human mitochondrial genome (NCBI accession number: NC_001807).

Forward1 (5′-CGCTACGACCAACTCATACAC- 3′) and Forward2 (5′-CGATTCCGCTACGACCAACTC- 3′),

Reverse1 (5′-TGGGTTCGATTCTCATAGTCCTAG- 3′) and Reverse2 (5′ -GATGGGTTCGATTCTCATAGTCC- 3′)

Products were amplified using Taq 2× Master Mix (New England Biolabs, Ipswich, Mass) according to the following parameters: Initial denaturing for 5 minutes at 95°C followed by 32 cycles of 30 s at 95°C, 30 s at 55°C, and 1 minute at 72°C. The final round was extended for an additional 5 minutes at 72°C. PCR products were separated on a 2% TAE-agarose gel, excised, and purified using the QIAquick gel extraction kit (Qiagen, Valencia, Calif). PCR products were directly sequenced by the Biotechnology Resource Lab at MUSC.

Retsriction Digest Analysis

Approximately 100 ng of amplified DNA was digested using *Taq*1 with the appropriate buffer (New England Bio-

labs, Ipswich, Mass). Products of the reaction were separated on a 2% TAE-agarose gel and visualized by ethidium bromide staining.

Cloning

PCR products were ligated into a PCR cloning vector, pGEMT® (Promega, Madison, Wis.). Plasmid DNA containing PCR products was transformed into TOP10 competent *E.coli* (Invitrogen) and positive recombinants were selected for by blue/white screening on LB/Ampicillin/IPTG/X-Gel plates incubated overnight at 37°C. Twelve colonies were picked for miniprep plasmid isolation (Qiaprep, Qiagen) and DNA sequencing. Eleven plasmids containing a PCR insert were sent for single-pass sequencing.

RESULTS

PCR amplified a product of approximately 206 bp in size. Direct sequencing of the PCR products did not reveal any mutation in the mtRNA-Ile. Restriction digest analysis with *Taq*1 did not result in cleavage of the PCR products. Of eleven clones that were sequenced, a single mutation was found in one clone at nucleotide 4327. The single T>C mutation was found at bp 4327 of the tRNA-Ile.

CONCLUSION AND IMPLICATIONS

Our results do not support our initial hypothesis. This patient does not carry the mutation at tRNA-Ile 4293. Restriction enzyme digestion of the PCR product should have produced restriction fragments of 150 and 54 bp in length, yet no fragments were detected. However, a mutation was revealed by DNA sequencing which may indicate a novel heteroplasmic mutation possibly linked to magnesium wasting in our patient. Because the 4327 bp mutation was discovered in less than 10% of the clones, we cannot yet rule out artifact.

Although hereditary single nucleotide polymorphisms have been implicated in disease progression in humans,[4] numerous studies suggest that aging may also play a role in producing mitochondrial mutations and degeneration.[5–8] It is also possible that additional mitochondrial mutations may exist that were not considered in this study. Future studies should include a more thorough examination of the mitochondrial genome to implicate other genes that may underlie hypomagnesemia.

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