



Mitochondrial transfer RNA mutations and hypertension

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ABSTRACT. Mutations in mitochondrial DNA have been found to be associated with hypertension. Of these, mitochondrial transfer RNA (mt-tRNA) is a hot spot for these pathogenic mutations. It is generally believed that these mutations may result in the failure of mt-tRNA metabolism, thereby worsening mitochondrial dysfunction and resulting in hypertension. mt-tRNA is known for its high frequency of polymorphisms and mutations, and the number of reports regarding mt-tRNA mutations and hypertension is increasing significantly. To better understand the molecular basis of maternally inherited hypertension, we reassessed the link between four mt-tRNA mutations (G15927A in tRNA^{Thr}, C7492T in tRNA^{Ser(UCN)}, A4386G in tRNA^{Gln}, and C14686T in tRNA^{Glu}) and hypertension. We first used the phylogenetic approach to investigate the deleterious roles of these mutations, then we used RNA Fold Web Server to predict the minimum free energy of these mt-tRNAs with and without mutations. Using the pathogenicity scoring system, we found that the G15927A and C7492T mutations are classified as pathogenic while all other studied mutations are neutral polymorphisms. Our study provides valuable information for the detection of pathogenic mt-tRNA mutations in hypertension.

Key words: Mitochondria; Mitochondrial tRNA mutations; Hypertension; Pathogenic mutations

INTRODUCTION

Hypertension is a leading cause of stroke, heart disease and kidney failure. Despite significant advances in our understanding of the pathophysiology of hypertension, it remains one of the world's greatest public health issues (El Shamieh et al., 2012). It is estimated that one-third of the world's adult population will be hypertensive by 2025 (Kearney et al., 2005). Therefore, understanding the etiology of hypertension has become a major focus in research, with particular emphasis on the genetic basis of hypertension. Human hypertension is a condition associated with endothelial dysfunction and oxidative stress (Romero and Reckelhoff, 1999), and mitochondrial dysfunction has been implicated in both human and experimental hypertension (Bernal-Mizrachi et al., 2005). Early and recent investigations showed significant maternal familial aggregation of high blood pressure, which suggested a contribution of the mitochondrial genome to hypertension (Bengtsson et al., 1979; DeStefano et al., 2001; Yang et al., 2007). An inefficient metabolism caused by mitochondrial dysfunction in skeletal and vascular smooth muscle may lead to the elevation of systolic blood pressure and the development of hypertension (Wilson et al., 2004; Arrell et al., 2006). Moreover, maternal transmission of hypertension has been observed in some pedigrees, suggesting that mutations in mitochondrial DNA (mtDNA) are the molecular basis of this disorder (Schwartz et al., 2004). In particular, the A4295G and A4263G mutations in the mitochondrial transfer RNA Ile (mt-tRNA^{Ile}) gene, and the A4401G mutation in the junction between the mt-tRNA^{Gln} and mt-tRNA^{Met} genes are associated with hypertension (Li et al., 2008, 2009; Wang et al., 2011). These mt-tRNA mutations may result in transcriptional and translational defects, resulting in mitochondrial respiratory chain dysfunction; however, a poor genotype-to-phenotype association is common, as is the case of the mt-tRNA^{Met} T4454C variant (Wang et al., 2014). Thus, determining pathogenic mutations and polymorphisms in mt-tRNA genes would be important for genetic counseling of patients with hypertension and their families.

In this study, we reassessed the association between four reported mt-tRNA mutations (G15927A in mt-tRNA^{Thr}, C7492T in mt-tRNA^{Ser(UCN)}, A4386G in mt-tRNA^{Gln}, and C14686T in mt-tRNA^{Glu}) and hypertension (Liu et al., 2014). The G15927A mutation has already been shown to be pathogenic and associated with coronary heart disease, and the cybrid cell model containing this mutation shows a significant reduction in steady-state levels of mt-tRNA^{Thr} (Jia et al., 2013). Thus, in this study, we focused on the other three mt-tRNA mutations. To do this, we first carried out the evolutionary conservation analysis for these mt-tRNA mutations. Next, we used the RNA Fold Web Server to predict the minimum free energy (MFE) of mt-tRNAs with and without these mutations. We also used the pathogenicity scoring system to discern whether these mt-tRNA mutations contribute to the genetic susceptibility of hypertension.

MATERIAL AND METHODS

Data extraction

To begin our study, we took the clinical, genetic and molecular datasets from a recent paper concerning the association between mt-tRNA mutations and hypertension (Liu et al., 2014). For comparison, we searched in Pubmed Central and other public domains for studies examining the association between these mt-tRNA mutations and clinical disorders.

Determining the degree of evolutionary conservation

With the purpose of understanding the role of mt-tRNA mutations in hypertension, we performed phylogenetic analysis for the conservation index (CI) of these mt-tRNA mutations. Briefly, 17 vertebrate mtDNA sequences were used for the inter-specific analysis. The CI is then calculated by comparing the human nucleotide variants with the other 16 vertebrates. Of note, a CI $\geq 70\%$ is considered as having functional potential.

Prediction of the secondary structure of mt-tRNA

We used the RNA Fold Web Server (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) to predict the MFE secondary structure of mt-tRNAs with and without these mutations. In addition to MFE folding, equilibrium base-pairing probabilities were calculated via John McCaskill's partition function (PF) algorithm (McCaskill, 1990; Gruber et al., 2008).

Pathogenicity scoring system for the mt-tRNA mutations

The pathogenicity scoring system proposed by Yarham et al. (2011) was applied to evaluate the total score of the candidate pathogenic mt-tRNA mutations. According to this system, a variant is classified as "definitely pathogenic" with a score ≥ 11 points, whereas a variant is defined as "possible pathogenic" with a score of 7-10 points and a "neutral polymorphism" has a score of 0-6 points.

RESULTS

Molecular features of mt-tRNA mutations associated with hypertension

Four mt-tRNA mutations have been found to be associated with hypertension. As shown in Figure 1, the G15927A (position 42) and C7492T (position 28) mutations are located in the anticodon stem of mt-tRNA^{Thr} and mt-tRNA^{Ser(UCN)}, respectively. The C14686T (position 61) mutation is located in the T-stem and the T4386C (position 15) mutation is located in the D-loop of the mt-tRNA^{Glu} gene (Florentz et al., 2003). A careful review of the literature indicates that the T4386C mutation is implicated in coronary heart disease (Qin et al., 2014). Moreover, the T4386C mutation has been found in patients with mitochondrial myopathy (Ueki et al., 2006).

Evolutionary conservation analysis of mt-tRNA mutations

Conservation assessment of the bases in the mt-tRNA gene suggests their evolutionary importance; therefore, it is anticipated that mutations may have pathological consequences. In this study, we performed the analysis of the CI of each mt-tRNA mutation using a phylogenetic approach. The CIs of C7492T, A4386G and C14686T are 73.1, 59.6 and 13.4%, respectively. Only the C7492T mutation has a high CI, suggesting that it may be involved in the pathogenesis of hypertension.

C7492T mutation alters mt-tRNA secondary structure

To test whether nucleotide alterations affect the structure of mt-tRNA, the sequences of wild type and mutant tRNAs harboring the C7492T, A4386G and C14686T were predicted using

the RNA Fold software. As displayed in Figure 2, except for the C7492T mutation, the mutations do not affect the secondary structure of the corresponding tRNAs, further indicating that the C7492T mutation may be involved in the pathogenesis of hypertension.

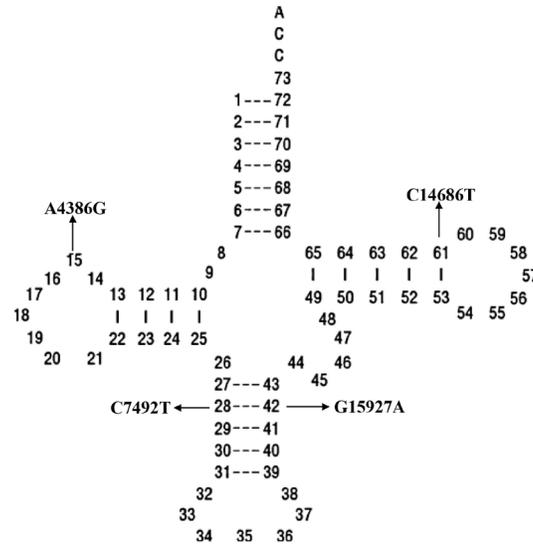


Figure 1. Cloverleaf structure of mt-tRNA with standard nucleotide numbering. The arrows indicate the positions of four mt-tRNA mutations: G15927A, C7492T, A4386G, and C14686T.

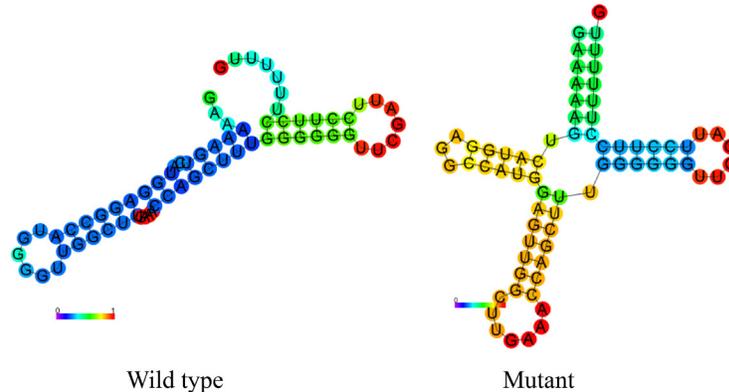


Figure 2. Prediction of the secondary structure of mt-tRNA^{Ser(UCN)} with and without the C7492T mutation.

Pathogenicity scoring system for the mt-tRNA mutations

According to the revised pathogenicity scoring system for mt-tRNA mutations (Yarham et al., 2011), we classified the C7492T mutation as “possibly pathogenic” with a total score of 7 points (Table 1). The scores of the other mutations were: A4386G = 5 points and C14686T = 3 points (data not shown). This indicates that they belong to the “neutral polymorphism” category.

Table 1. Pathogenicity scoring system for the C7492T mutation.

| Scoring criteria | C7492T mutation | Score/20 | Classification |
|--|-----------------|----------|---------------------|
| More than one independent report | Yes | 2 | |
| Evolutionary conservation of the base pair | One change | 2 | |
| Variant heteroplasmy | No | 0 | |
| Segregation of the mutation with disease | Yes | 2 | |
| Histochemical evidence of mitochondrial disease | No evidence | 0 | |
| Biochemical defect in complex I, III or IV | No | 0 | |
| Evidence of mutation segregation with biochemical defect from single-fiber studies | No | 0 | |
| Mutant mt-tRNA steady-state level or evidence of pathogenicity in trans-mitochondrial cybrid studies | Weak evidence | 1 | |
| Total score | | 7 | Possibly pathogenic |

Scoring system: ≤6 points: neutral polymorphisms; 7-10 points: possibly pathogenic; ≥11 points: definitely pathogenic.

DISCUSSION

mt-tRNA point mutations are increasingly becoming recognized as important in disease, with novel pathogenic mutations being reported in all mt-tRNAs. Today, approximately 200 pathogenic mutations have been mapped to mt-tRNA genes (<http://www.mitomap.org/MITOMAP>), emphasizing the importance of mt-tRNA for mitochondrial function. It should be kept in mind that each cell contains hundreds to thousands of copies of mtDNA. Even in healthy individuals, a small population of polymorphic mutations is found in mtDNA. In fact, the Mitomap database includes a considerable amount of data on polymorphic mutations (McFarland et al., 2004). If a mutation in the mt-tRNA gene does not affect mtDNA replication or transcription, it may instead have a negative effect on the biogenesis and function of mt-tRNAs post-transcriptionally, whether it is in processing, post-transcriptional modification, aminoacylation or interactions with the mitoribosome during translation (Suzuki et al., 2011). Distinguishing the polymorphisms and mutations in mt-tRNAs is important and will lead to improved diagnosis and genetic counseling.

The current study investigates the possible link between four reported mt-tRNA mutations and hypertension. The G15927A mutation has already been identified as a pathogenic mutation, as it causes ~80% reduction in the steady-state level of mt-tRNA^{Thr}. Moreover, *in vivo* mitochondrial protein labeling analysis showed ~53% reduction in the rate of mitochondrial translation in mutant cells (Jia et al., 2013). The homoplasmic A4386G and C14686T mutations occur at positions that are not evolutionarily conserved and do not cause alterations in mt-tRNA secondary structure. In addition, the C14686T mutation occurs at position 61 in the T-loop of tRNA^{Glu} and has not been reported in Mitomap or mtDB, suggesting that it is a rare variant with allele frequencies less than 0.5%. By contrast, the C7492T mutation is reported to increase the penetrance and expressivity of deafness associated with the 12S rRNA A1555G mutation (Lu et al., 2010). In the case of the C7492T mutation, phylogenetic conservation analysis supports that this mutation is conserved in different species and RNA Fold results show that it causes a change in the MFE of tRNA^{Ser(UCN)}. This indicates that it may cause pathogenicity by disrupting the formation of the anticodon stem, which may in turn decrease the efficiency of codon-anticodon recognition and aminoacylation.

In summary, this is the first study to assess the association between the C7492T mutation and hypertension, and our data shows that this mutation is “possibly pathogenic” in hypertension. However, owing to the limitations of this study, further investigation using appreciate methods, such as a cybrid cell containing this mutation and determination of the mt-tRNA steady-state level, are required to evaluate the role of the C7492T mutation in hypertension.

Conflicts of interest

The authors declare no conflict of interest.

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