Leber’s Hereditary Optic Neuropathy: the roles of mitochondrial transfer RNA variants

Yu Ding¹, Guangchao Zhuo¹, Qinxian Guo¹ and Meiya Li²

¹Central laboratory, Hangzhou First People’s Hospital, Hangzhou, Zhejiang, China
²Academy of Chinese Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China

ABSTRACT

Leber’s Hereditary Optic Neuropathy (LHON) was a common maternally inherited disease causing severe and permanent visual loss which mostly affects males. Three primary mitochondrial DNA (mtDNA) mutations, ND1 3460G>A, ND4 11778G>A and ND6 14484T>C, which affect genes encoding respiratory chain complex I subunit, are responsible for >90% of LHON cases worldwide. Families with maternally transmitted LHON show incomplete penetrance with a male preponderance for visual loss, suggesting the involvement of secondary mtDNA variants and other modifying factors. In particular, variants in mitochondrial tRNA (mt-tRNA) are important risk factors for LHON. These variants decreased the tRNA stability, prevent tRNA aminoacylation, influence the post-transcriptional modification and affect tRNA maturation. Failure of mt-tRNA metabolism subsequently impairs protein synthesis and expression, folding, and function of oxidative phosphorylation (OXPHOS) enzymes, which aggravates mitochondrial dysfunction that is involved in the progression and pathogenesis of LHON. This review summarizes the recent advances in our understanding of mt-tRNA biology and function, as well as the reported LHON-related mt-tRNA second variants; it also discusses the molecular mechanism behind the involvement of these variants in LHON.

INTRODUCTION

Leber’s Hereditary Optic Neuropathy (LHON) is named after Theodore Leber, a German ophthalmologist who first described the defining clinical features of this disorder in 1871. LHON is the commonest maternally inherited eye diseases, which typically affects young adults, with most of patients being males (Sandbach & Newman, 2001; Man, Turnbull & Chinnery, 2002). Vision loss from LHON results from selective degeneration of retinal ganglion cells (RGCs) (Carelli et al., 2009). Loss of RGCs occurs in around 50% of male and but only in approximately 10%～15% of female patients. It causes adult-onset progressive and painless visual loss which begins in only one eye, but usually manifests in the other eye within a few weeks. Eventually, visual acuity in both eyes deteriorated to 20/200 or worse. Moreover, LHON patients may exhibit abnormal symptoms, including movement...
disorders, dystonia or multiple sclerosis like symptoms, which pose a significant challenge for clinicians (Yu-Wai-Man, Griffiths & Chinnery, 2011; Jia et al., 2006).

The prevalence of LHON has been well established in Northern European populations, with figures ranging from one in 30,000 to one in 50,000 (Man, Turnbull & Chinnery, 2002; Rosenberg et al., 2016; Yu-Wai-Man et al., 2003), and one in 1,000,000 in Japanese population according to a recent survey (Ueda et al., 2017). Clinically, over 90% of LHON cases are caused by one of three mtDNA missense mutations in genes encoding subunits of NADH dehydrogenase (ND): ND1 3460G>A, ND4 11778G>A and ND6 14484T>C (Wallace et al., 1988; Catarino et al., 2017; Huoponen et al., 1991). Although the genetic basis of LHON was remains unclear, it has become apparent that mitochondrial dysfunction caused by mtDNA mutations is the molecular basis of this disease. mt-tRNA genes are also highly susceptible to point mutations, which are primary causes of mitochondrial dysfunction (Scaglia & Wong, 2008). It is thus possible that mt-tRNA variants also play important roles in the phenotypic manifestation of LHON-associated primary mutations.

In this review, we cover the basic aspects of mitochondrial biology and genetics, as well as mt-tRNA maturation, and summarize the mt-tRNA variants that have been reported to be associated with LHON.

**REVIEW METHODOLOGY**

We carried out a search in PubMed Central (http://www.ncbi.nlm.nih.gov) and other public domains with the following keywords: “mitochondrial biology”, “mtDNA genetics”, “mt-tRNA function”, “mt-tRNA maturation”, “mt-tRNA end processing”, “mt-tRNA modification”, “mt-tRNA variants and LHON” (last search update on October 8, 2020). The “OR” and “AND” terms were used for the various searches. We excluded studies if the crucial data were not reported in the original papers, or if there was a very high likelihood of inaccurate reporting.

To investigate the candidate pathogenic mt-tRNA variants, the Mamit-tRNA database (http://mamit-tRNA.u-strasbg.fr) was used to locate the positions of the mt-tRNA variants, as well as the cloverleaf structure of tRNAs (Pütz et al., 2007). Additionally, the conversion of nucleotide numbering in human mt-tRNA genes was based on the criteria proposed by Andrews et al. (1999). The conservation index (CI) of each reported mt-tRNA variant was analyzed by the ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Hall, 2013).

**Mitochondrial biology and genetics**

Mitochondria originated from within the bacterial phylum α-Proteobacteria and became established via an endosymbiotic event (Lane & Martin, 2010). Mitochondria are critical organelles that perform a remarkably diverse set of cellular functions. The most important of these is the generation of ATP via OXPHOS, but mitochondria also play critical roles in the regulation of apoptosis, maintenance of cellular redox homeostasis and intracellular calcium signaling (Duchen, 2004; Tait & Green, 2010; Sena & Chandel, 2012; Rizzuto et al., 2012).
The human mitochondrial genomes (mitogenomes) are circular, 16,569-bp in length, and contain 37 genes encoding 13 proteins required for OXPHOS and the electron transport chain (ETC) (Fig. 1) (Andrews et al., 1999). mtDNA also encodes RNAs, which are involved in the translation of ETC proteins (Luo et al., 2018). Owing to its location within the mitochondrial matrix, lack of protective histone wrapping, as well as a comparatively limited repair mechanism, mtDNA is more vulnerable to oxidative modifications which accumulate over time (Yakes & Van Houten, 1997). Indeed, it has been shown that mtDNA has a significantly higher mutation rate than nuclear DNA (Neckelmann et al., 1987).

Unlike nuclear DNA, in which there are only two copies of each gene per cell, thousands of copies of mtDNA are presented in every cell. Typically, individuals harbor only one mtDNA genotype, and all mitogenomes are genetically identical, a condition called homoplasy. This contrasts with heteroplasmic, which involves the presence of a mixture of mutant and wild-type mtDNA genomes within a cell. Through somatic mutagenesis and ongoing replication of mtDNA, mutations can clonally expand through either random drift or selective processes, and become present at varying proportions or degrees of heteroplasmy with cells (Elson et al., 2001). Among families affected by LHON, 85%–90% of carriers are homoplasmic for mtDNA mutation. However, some studies have indicated
that mtDNA heteroplasmy may be a factor determining the penetrance of LHON (Li et al., 2019; Finsterer & Zarrouk-Mahjoub, 2018). In certain families, rapid segregation of the mitochondrial genotype toward mutant-type homoplasmy of either 11778G>A (Bolhuis et al., 1990) or 3460G>A mutation in blood (Black et al., 1996) has been shown to be associated with the development of LHON in later generations. It has been suggested that the risk of disease conversion is low if the mutational load is below the threshold of 60% (Chinnery et al., 2001). Although it is not possible to accurately predict whether an LHON carrier will eventually lose vision, individuals can be counseled based on the two major identifiable risk factors in this disorder: age and sex.

**Three LHON-associated primary mutations**

The majority of patients with LHON harbor one of three primary mtDNA point mutations: 3460G>A (Howell et al., 1991; Huoponen et al., 1991), 11778G>A (Wallace et al., 1988), and 14484T>C (Johns, Neufeld & Park, 1992; Mackey & Howell, 1992). They are found exclusively in families affected by LHON and never in control subjects. The G to A transition at position 11778 converts a conserved arginine to histidine, has been associated with poor visual outcome and prognosis (Newman, Lott & Wallace, 1991). Meanwhile, the 3460G>A mutation causes the alteration of a highly conserved alanine to threonine, which is present in around 15% of LHON families (Howell, et al. 1991). Moreover, the T to C transition at nucleotide 14484 in ND6 (methionine to valine) has been shown to be tightly linked to the LHON phenotype (Johns, Neufeld & Park, 1992). Interestingly, younger age at onset (<15 years) and mutation type appear to dictate visual outcome; patients with the 14484T>C mutation have a better visual prognosis with 60% attaining some visual improvement compared with only 5% of those carrying the 11778G>A mutation.

The incomplete penetrance, high male to female ratio, and existence of LHON plus cases strongly suggest the involvement of modifying factors such as genetic or environmental ones (Tońska, Kodroń & Bartnik, 2010; Caporali et al., 2017). In particular, genetic factors such as mt-tRNA variants can play active roles in the phenotypic manifestation of LHON-associated primary mutations.

**Nuclear genes**

Although the mitochondrial proteome consists of over 1000 proteins, only 14 of them are encoded by mtDNA. Thus, the nuclear genome encodes >90% of peptides involved in the OXPHOS system (Leigh-Brown, Enriquez & Odom, 2010). Moreover, incomplete penetrance and male bias in patients with LHON suggest that an X-linked modified gene is necessary for the disease expression (Bu & Rotter, 1991). A recent genome-wide study of 1281 Chinese probands with LHON identified a novel LHON susceptibility allele (c.157C>T, p. Arg53Trp) in the PRICKLE3 gene, which links to ATPase biogenesis manifested LHON (Yu et al., 2020). Moreover, a missense mutation in YARS2 (c.572G>T, p. Gly191Val) was shown to interact with the 11778G>A mutation to cause visual failure (Jiang et al., 2016).
mt-tRNA structure and function

mt-tRNA is a short, non-coding RNA that constitutes approximately 4~10% of all cellular RNAs (Kirchner & Ignatova, 2015). In fact, most mt-tRNAs from all domains of life have a highly conserved cloverleaf structure, consisting of an acceptor arm, D-arm, anticodon stem, variable region, and TψC loop, with an average length of 73 nucleotides (nts). However, mt-tRNA genes encode transcripts that show considerable deviation of this standard, having a reduced D-arm or TψC loop or even completely lacking one of these elements (e.g., tRNA\text{Ser(AGY)}), resulting in tRNAs as small as 66 nts (Hanada et al., 2001). In addition, mt-tRNA\text{Ser(UCN)} has several distinct structural features, including only one base (A9) between the acceptor arm and D-arm, a short D-loop, a variable region, and an extended anticodon stem with 6-bp (Watanabe et al., 1994).

As adapter molecules to convert the information stored in amino acid (AA) sequences, tRNAs play a central role in protein synthesis (Stowe & Camara, 2009). Although tRNAs comprise only around 10% of the total coding capacity of the mitogenomes, more than half of mtDNA mutations causing diseases are located in mt-tRNA genes (https://www.mitomap.org/MITOMAP) (Taanman, 1999), emphasizing the importance of tRNAs for mitochondrial function.

tRNA end processing

The excision of tRNAs from primary polycistronic mitochondrial transcripts is catalyzed by two specialized enzymes, RNase P and tRNase Z (Fig. 2). RNase P is an endonuclease that catalyzes the cleavage of the 5′leader sequence from pre-tRNA transcripts (Rossmannith et al., 1995). Human mitochondrial RNase P (mtRNase P) is a RNase P complex consisted of three proteins, called MRPP1; MRPP2 and MRPP3 (Holzmann et al., 2008). In fact, human mtRNase P cleaves a wide range of tRNA precursors in vitro (Rossmannith, 1997; Rossmannith et al., 1995), and its activity is detectable even in crude mitochondrial extracts and thereby appears to be relatively abundant (Rossmannith et al., 1995). At the other end, 3′trailers are removed by the endonuclease tRNase Z (Phizicky & Hopper, 2010; Hartmann et al., 2009; Marai & Lamichhane, 2011). Both short and long forms of tRNase Z are present in eukaryotes, designated tRNase ZS (280 to 360 AAs) and tRNase ZL (750 to 930 AAs) respectively (Jshii et al., 2005; Li de la Sierra-Gallay, Pellegrini & Condon, 2005). The C-terminal part of tRNase ZL has sequence homology with tRNase ZS. However, in contrast to the single mechanism of 5′leader removal, 3′trailers can also be removed by one or more 3′exoribonucleases (Rex1p, and perhaps others) (Copela et al., 2008; Ozanick et al., 2009). The 5′-before-3′end processing appears to apply most clearly when tRNase Z is used for 3′processing.

tRNA post-transcriptional modifications

For mt-tRNA maturation, post-transcriptional modifications, together with the 5′and 3′nucleolytic excision from precursor RNAs are required. Certain modifications are necessary for maintenance of mt-tRNA structure and steady-state level, as well as ensuring the efficiency of protein synthesis during mitochondrial translation. Up to date, more than 30 modified mt-tRNA positions have been reported (Suzuki, Nagao & Suzuki,
Modifications cluster occurs at two main regions of tRNA molecule: the structural core and the anticodon stem. Chemical modifications in the structural region are relatively simple, for instance, methylation, pseudouridylation and dihydrouridylation. Furthermore, modifications in the anticodon stem of mt-tRNAs include methylation and pseudouridylation, in several cases, with more complex additions, specially the modifications at positions 34 and 37 (El Yacoubi, Bailly & Crécy-Lagard, 2012). Four types of modified nucleotides were found at the wobble positions of 10 tRNA species that correspond to two codon sets. The modifications consisted of 5-formylcytidine at the wobble position of tRNA\textsuperscript{Met} (Bilbille et al., 2011), queuosine at the wobble positions of four tRNA\textsuperscript{Tyr}, tRNA\textsuperscript{His}, tRNA\textsuperscript{Asn} and tRNA\textsuperscript{Asp} (Iwata-Reuyl, 2008). In addition, five tRNAs were found to have taurine-containing uridines 5-taurinomethyluridine was identified in the tRNA\textsuperscript{Leu(UUR)} and tRNA\textsuperscript{Trp}, and 5-taurinomethyl-2-thiouridine in tRNA\textsuperscript{Lys}, tRNA\textsuperscript{Glu} and tRNA\textsuperscript{Gln} (Nagao et al., 2009; Suzuki et al., 2002).

**tRNA aminoacylation**

Aminoacyl-tRNA synthetases (aaRSs), encoded by nuclear genes, play essential roles in protein synthesis. To start this process, aaRSs must catalyze the attachment of AAs to the corresponding tRNAs (Yao & Fox, 2013). This biochemical reaction requires the following steps: 1. AA+ATP $\rightarrow$ aminoacyl-AMP+PPi; 2. aminoacyl-AMP + tRNA $\rightarrow$ aminoacyl-tRNA+AMP. Today, nineteen species of aaRS genes were annotated in
the human genome database (Antonellis & Green, 2008). Mammalian mitochondria had no enzyme corresponding to glutaminyl-tRNA synthetase (GlnRS) (Nagao et al., 2009). Mitochondrial LysRS and GlyRS were encoded by the same genes as the cytoplasmic LysRS and GlyRS, respectively, whereas the other aaRSs were encoded by genes different from the cytoplasmic ones (Ling, Reynolds & Ibba, 2009).

Secondary mt-tRNA variants

Although most LHON cases are caused by one of three pathogenic mtDNA mutations, no primary mutations are identified in a minority of LHON patients, these other homoplastic mtDNA are considered as secondary variants that can be responsible for disease phenotype variation and different penetrance having synergistic effects with the primary mtDNA mutations (Bosley & Abu-Amero, 2010; Zhang et al., 2009) (Fig. 4 and Tables 1 and 2).
Figure 4  Secondary structure of (A) mt-tRNA\textsubscript{Phe}, (B) tRNA\textsubscript{Leu(UUR)}, (C) tRNA\textsubscript{Met}, (D) tRNA\textsubscript{Ala}, (E) tRNA\textsubscript{His}, (F) tRNA\textsubscript{Glu}, (G) tRNA\textsubscript{Thr} and (H) tRNA\textsubscript{Pro}.

Full-size DOI: 10.7717/peerj.10651/fig-4

mt-tRNA variants enhance the phenotypic expression of the primary mtDNA mutations

\textit{tRNA\textsubscript{Phe} variant}

According a recent experimental study, the 593T>C variant appears a high frequency in LHON patients (Ji et al., 2008). This variant occurs at the D-arm of tRNA\textsubscript{Phe} and decreases the free energy (Zhang et al., 2011). Moreover, the electrophoretic mobility of the tRNA\textsubscript{Phe} gene with or without 593T>C transcribes confirm the change of secondary structure. Thus, the 593T>C variant may have a synergistic effect with the LHON-related 11778G>A mutation. By using lymphoblastoid cell lines derived from a Chinese family, an approximately ∼46% decrease in the steady-state level of tRNA\textsubscript{Phe} was identified in mutant cell lines. Western blotting analysis showed an approximately 35% reduction in the levels of mitochondrial translation in mutant cell lines carrying the 593T>C variant (Chen et al., 2017). Interestingly, the 593T>C variant is suggested to increase the penetrance and expressivity of LHON-associated ND6 14484T>C mutation in one Chinese pedigree (Man et al., 2020)

\textit{tRNA\textsubscript{Met} variant}

The 4435A>G variant affects a highly conserved adenosine at position 37, 3’ adjacent to the tRNA\textsubscript{Met} anticodon, which is important for the fidelity of codon recognition and stabilization (Lu et al., 2011). This variant has been found to modulate the clinical expression of LHON-associated ND4 11778G>A mutation in a Chinese family (Qu et al.,
Table 1: Characterization of 22 human mt-tRNAs.

| tRNA Species | Starting | Ending | Length (bp) |
|--------------|----------|--------|-------------|
| tRNA^{Phe}   | 577      | 647    | 71          |
| tRNA^{Val}   | 1,602    | 1,670  | 69          |
| tRNA^{Leu(UUR)} | 3,230 | 3,304  | 75          |
| tRNA^{Glu}   | 4,329    | 4,400  | 72          |
| tRNA^{Met}   | 4,402    | 4,469  | 68          |
| tRNA^{Trp}   | 5,512    | 5,579  | 68          |
| tRNA^{Ala}   | 5,587    | 5,655  | 69          |
| tRNA^{Asn}   | 5,657    | 5,729  | 73          |
| tRNA^{Gly}   | 5,761    | 5,826  | 66          |
| tRNA^{Glu}^{5UCN} | 5,826 | 5,891  | 66          |
| tRNA^{Ser(UCN)} | 7,446 | 7,514  | 69          |
| tRNA^{Asp}   | 75,18    | 7,585  | 68          |
| tRNA^{Thr}   | 8,295    | 8,364  | 70          |
| tRNA^{Glu}   | 9,991    | 10,058 | 68          |
| tRNA^{Arg}   | 10,405   | 10,469 | 65          |
| tRNA^{His}   | 12,138   | 12,206 | 69          |
| tRNA^{Ser(AGY)} | 12,207 | 12,265 | 59          |
| tRNA^{Leu(UUN)} | 12,266 | 12,336 | 71          |
| tRNA^{Glu}   | 14,674   | 14,742 | 69          |
| tRNA^{Thr}   | 15,888   | 15,953 | 66          |
| tRNA^{Pro}   | 15,956   | 16,023 | 68          |

In fact, the 4435A>G variant introduces a tRNA methyltransferase 5 (TRMT5)-catalyzed m1G37 modification of tRNA^{Met}. Functional analysis of cybrid cells harboring this variant indicated significantly decreased efficiency in aminoacylation and steady-state levels of tRNA^{Met}, compared with findings in control cybrids (Zhou et al., 2018). An approximately 40% reduction in the level of tRNA^{Met} was observed in cells carrying the 4435A>G variant. The failure in tRNA metabolism, caused by the 4435A>G variant, led to an approximately 30% reduction in the rate of mitochondrial translation (Liu et al., 2009). These results indicate that the 4435A>G variant may lead to defects in mt-tRNA modification and enhance the phenotypic expression of LHON-related ND4 11778G>A mutation.

**tRNA^{Ala} variant**

According to recent report, the tRNA^{Ala}5601C>T variant is associated with LHON ((Ding et al., 2020)). The homoplasmic 5601C>T variant has been reported in several LHON-affected pedigrees and one pedigree affected with hypertension (Zhou et al., 2012; Zheng et al., 2018; Ding et al., 2020; Zheng et al., 2018). This variant is located at very conserved nucleotide (position 59) in the TψC loop of tRNA^{Ala}, and creates a novel Watson-Crick base-pairing (55T-59C). Bioinformatic analysis revealed that 5601C>T alters the secondary structure...
| tRNA species | Allele | Position | Structural location | Homoplasmy or Heteroplasmy | Aberrant tRNA biology | References |
|--------------|--------|----------|---------------------|---------------------------|------------------------|------------|
| tRNA<sub>Phe</sub> | 593T>C  | 17       | D-arm               | Homoplasmy                | Reduced expression of functional tRNA | Ji et al. (2008), Zhang et al. (2011) |
| tRNA<sub>Leu(UUR)</sub> | 3275C>T | 44       | Variable region     | Homoplasmy                | Disrupt conserved base pairing | Garcia-Lozano et al. (2000), Ding et al. (2018) |
| tRNA<sub>Met</sub> | 4435A>G  | 37       | Anticodon stem      | Homoplasmy                | Introduce the m<sup>1</sup>G37 modification | Qu et al. (2006), Zhou et al. (2018) |
| tRNA<sub>Ala</sub> | 5587T>C  | 73       | Acceptor arm        | Heteroplasmy              | Affect the 3′end processing | Ji et al. (2017), Tang et al. (2010) |
|               | 5601C>T  | 59       | T ψC loop           | Homoplasmy                | Create conserved base pairing | Ding et al. (2020) |
| tRNA<sub>His</sub> | 12192G>A | 59       | T ψC loop           | Homoplasmy                | Disrupt conserved base pairing | Mimaki et al. (2003), Ding et al. (2019) |
| tRNA<sub>Glu</sub> | 14693A>G | 54       | T ψC loop           | Homoplasmy                | Defect in taurine modification | Tong et al. (2007), Zhang et al. (2010) |
| tRNA<sub>Thr</sub> | 15927G>A | 42       | Anticodon stem      | Homoplasmy                | Disrupt conserved base pairing | Zhang et al. (2018), Jia et al. (2013) |
|               | 15951A>G | 71       | Acceptor arm        | Homoplasmy                | Disrupt conserved base pairing | Li et al. (2006), Lyu et al. (2019) |
| tRNA<sub>Pro</sub> | 15986insG | 39      | Anticodon stem      | Homoplasmy                | Disrupt conserved base pairing | Jancic et al. (2020) |
of tRNAAla, thus, this variant contributes to the structural formation and stabilization of functional tRNAAla and leads to mitochondrial dysfunction caused by 11778G>A mutation.

**tRNAHis variant**
The 12192G>A variant, combined with the ND4 11778G>A mutation, has been reported in patients with both LHON and cardiomyopathy (Mimaki et al., 2003). Interestingly, the 12192G>A variant occurs 2-bp from the 3’ end of the TψC loop of tRNAHis, which is highly conserved from various species (Pütz et al., 2007), and is believed to be involved in tertiary interaction between the TψC loop and the truncated D-arm (Ueda et al., 1992). Biochemical analysis of polymononuclear leukocytes (PMNs) which containing the 12192G>A variant showed a significant decrease in ATP production and an increased in ROS generation (Ding et al., 2019), suggesting that this polymorphism increases the penetrance and expressivity of LHON.

**tRNAGlu variant**
The homoplasmic 14693A>G variant in the TψC loop of tRNAGlu suggested to modulate the phenotypic manifestation of LHON-associated ND1 3460G>A mutation in a Chinese pedigree (Tong et al., 2007). This variant has also been found in three LHON-affected families (Zhang et al., 2010). In fact, the 14693A>G variant is considered to be associated with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (Tzen et al., 2003), PCOS (Ding et al., 2017), diabetes mellitus (Tang et al., 2006), and hearing loss (Ding et al., 2009). At the molecular level, the 14693A>G variant is located at very conserved nucleotide of tRNAGlu(conventional position 54), the nucleotide at that position in the TψC loop is often modified and contributes to the structural formation and stabilization of functional tRNAs (Paris & Alfonzo, 2018). Therefore, the change of structure of tRNAs due to this variant may lead to a failure in tRNA metabolism, which would in turn impair of mitochondrial translation.

**tRNAPro variant**
Recently, a novel mutation (15986insG) in mt-tRNAPro was identified in a Serbian family with LHON-associated 3460G>A mutation (Jancic et al., 2020). This insertion occurs at the anticodon stem of tRNAPro, which disrupts a very conserved Watson-Crick base-pairing (31G-39C). In fact, tRNAIle 4298G>A occurring at the same position has been regarded as a pathogenic mutation associated with chronic progressive external ophthalmoplegia (CPEO) (Taylor et al., 1998). Thus, it can be speculated that 15986insG, which is similar to the 4298G>A mutation, may also lead to mitochondrial dysfunction that modulates the phenotypic expression of LHON-related 3460G>A mutation.

**Other reported mt-tRNA variants**

**tRNALeu(UUR) variant**
According to a report by Garcia-Lozano et al. (2000), the 3275C>T variant in tRNALeu(UUR) contributes to the clinical expression of LHON and is associated with metabolic syndrome (MetS) and polycystic ovary syndrome (PCOS) (Ding et al., 2018). In fact, the homoplasmic 3275C>T variant disrupts a highly evolutionary conserved base-pairing (28A-46C) in the
variable region of tRNA\textsuperscript{Leu(UUR)} (Salinas-Giegé, Giegé & Giegé, 2015), and bioinformatic analysis has revealed that the 3275C>T variant causes the thermodynamic change of tRNA\textsuperscript{Leu(UUR)}. Moreover, patients with this variant have a lower level of mitochondrial membrane potential (MMP), ATP and mtDNA copy number, and higher ROS than the controls (Ding et al., 2018). Thus, the 3275C>T variant may lead to mitochondrial dysfunction, which is involved in the pathogenesis of LHON.

**tRNA\textsuperscript{Ala} variant**

The tRNA\textsuperscript{Ala}5587T>C variant is reported to be associated with LHON according to a recent study (Ji et al., 2017). The heteroplasmic 5587T>C variant occurs at the end of the tRNA\textsuperscript{Ala} and may alter the tertiary structure of this tRNA (position 73), this nucleotide position is extremely conserved from bacteria to human mitochondria. Thus, it can be speculated that this variant influences the 3’ end sequences of the acceptor arm of tRNA\textsuperscript{Ala}, subsequently affecting the efficiency of protein translation. Furthermore, the 5587T>C variant has been shown to be associated with progressive unstable gait, dysarthria, hearing loss, muscle cramps and myalgia (Tang et al., 2010; Crimi et al., 2002).

**tRNA\textsuperscript{Thr} variants**

The tRNA\textsuperscript{Thr} gene is a “hot” spot for genetic variants associated with LHON, these variants included 15951A>G and 15927G>A (Lyu et al., 2019). Notably, the 15951A>G variant is localized at adjacent to 3’end (position 71) of tRNA\textsuperscript{Thr}, the adenine at this position is highly conserved from bacteria to human mitochondria (Helm et al., 2000). This nucleotide at position 71 of tRNAs has been shown to play an important role in the recognition by their cognate aaRS (Florentz et al., 2003). Furthermore, compared with controls, cybrid cells containing the 15951A>G variant showed an approximately ~35% reduction in the level of tRNA\textsuperscript{Thr}, the failure in tRNA metabolism resulting from this variant may lead to the impairment of mitochondrial translation (Li et al., 2006).

Moreover, the well-known 15927G>A variant disrupts a conservative base-pairing (28C-42G) in the anticodon stem of tRNA\textsuperscript{Thr}. This variant was shown to be associated with an approximately ~60% reduction in the level of tRNA\textsuperscript{Thr} in cybrid cells (Zhang et al., 2018; Jia et al., 2013). Additionally, western blot analysis showed the variable reductions of four mtDNA-encoded proteins in association with the variant, with especially marked decreases of ND1 and CYTB (Wang et al., 2008). Furthermore, the 15927G>A variant was found to result in significantly reduced activities of Complexes I and III, as observed in cybrid cells (Zhang et al., 2018). Notably, the 15927G>A variant has also been reported to be associated with hearing loss (Ding et al., 2019; Wang et al., 2008) and coronary heart disease (Jia et al., 2019).

**CONCLUSIONS**

Mitochondrial dysfunction and mtDNA genetic variants are linked to LHON. In previous studies, we noted that mainly LHON-associated pathogenic mtDNA mutations are located in genes encoding respiratory chain Complex I subunits. Moreover, secondary mt-tRNA variants may have synergistic effects on the clinical expression of LHON. In
fact, mt-tRNA variants have structural and functional effects, including altering the tRNA secondary structure and the processing of tRNA precursors, reducing tRNA steady state level, and causing the defects in tRNA modifications. These events would exacerbate the mitochondrial dysfunction caused by the three primary mutations. Therefore, our findings are valuable for the further deepening our understanding of the pathophysiology and management of LHON.

ACKNOWLEDGEMENTS

We would like the members of our laboratory for useful comments for this review. We thank Liwen Bianji, Edanz Group China, for editing the English text of a draft of this manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work was supported by grants from Ministry of Public Health from Zhejiang Province (no. 2018ZH019 and 2021RC022), and the Zhejiang Provincial Administration of Traditional Chinese Medicine (no. 2018ZB082). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
Ministry of Public Health from Zhejiang Province: 2018ZH019, 2021RC022.
Zhejiang Provincial Administration of Traditional Chinese Medicine: 2018ZB082.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Yu Ding conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Guangchao Zhuo analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
• Qinxian Guo analyzed the data, prepared figures and/or tables, and approved the final draft.
• Meiya Li conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
This is a literature review, therefore there is no raw data.
REFERENCES

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics* 23(2):147 DOI 10.1038/13779.

Antonellis A, Green ED. 2008. The role of aminoacyl-tRNA synthetases in genetic diseases. *Annual Review of Genomics and Human Genetics* 9:87–107 DOI 10.1146/annurev.genom.9.081307.164204.

Bilbille Y, Gustilo EM, Harris KA, Jones CN, Lusic H, Kaiser RJ, Delaney MO, Spremulli LL, Deiters A, Agris PF. 2011. The human mitochondrial tRNAMet: structure/function relationship of a unique modification in the decoding of unconventional codons. *Journal of Molecular Biology* 406(2):257–274 DOI 10.1016/j.jmb.2010.11.042.

Black GC, Morten K, Laborde A, Poulton J. 1996. Leber’s hereditary optic neuropathy: heteroplasmy is likely to be significant in the expression of LHON in families with the 3460 ND1 mutation. *British Journal of Ophthalmology* 80(10):915–917 DOI 10.1136/bjo.80.10.915.

Bolhuis PA, Bleeker-Wagemakers EM, Ponne NJ, Van Schooneveld MJ, Westerveld A, Van den Bogert C, Tabak HF. 1990. Rapid shift in genotype of human mitochondrial DNA in a family with Leber’s hereditary optic neuropathy. *Biochemical and Biophysical Research Communications* 170(3):994–997 DOI 10.1016/0006-291x(90)90490.

Bosley TM, Abu-Amero KK. 2010. Assessing mitochondrial DNA nucleotide changes in spontaneous optic neuropathies. *Ophthalmic Genetics* 31(4):163–172 DOI 10.3109/13816810.2010.514015.

Bu XD, Rotter JI. 1991. X chromosome-linked and mitochondrial gene control of Leber hereditary optic neuropathy: evidence from segregation analysis for dependence on X chromosome inactivation. *Proceedings of the National Academy of Sciences of the United States of America* 88(18):8198–8202 DOI 10.1073/pnas.88.18.8198.

Caporali L, Maresca A, Capristo M, Del Dotto V, Tagliavini F, Valentino ML, La Morgia C, Carelli V. 2017. Incomplete penetrance in mitochondrial optic neuropathies. *Mitochondrion* 36:130–137 DOI 10.1016/j.mito.2017.07.004.

Carelli V, La Morgia C, Valentino ML, Barboni P, Ross-Cisneros FN, Sadun AA. 2009. Retinal ganglion cell neurodegeneration in mitochondrial inherited disorders. *Biochimica Et Biophysica Acta* 1787(5):518–528 DOI 10.1016/j.bbabio.2009.02.024.

Catarino CB, Ahting U, Gusic M, Iuso A, Repp B, Peters K, Biskup S, Livonius Bvon, Prokisch H, Klopstock T. 2017. Characterization of a Leber’s hereditary optic neuropathy (LHON) family harboring two primary LHON mutations m.11778G>A and m.14484T>C of the mitochondrial DNA. *Mitochondrion* 36:15–20 DOI 10.1016/j.mito.2016.10.002.

Chen X, Nie Z, Wang F, Wang J, Liu XW, Zheng J, Guo YF, Guan MX. 2017. Late onset nonsyndromic hearing loss in a Dongxiang Chinese family is associated with the
593T>C variant in the mitochondrial tRNAPhe gene. *Mitochondrion* **35**:111–118 DOI 10.1016/j.mito.2017.05.013.

Chinnery PF, Andrews RM, Turnbull DM, Howell NN. 2001. Leber hereditary optic neuropathy: does heteroplasmy influence the inheritance and expression of the G11778A mitochondrial DNA mutation?. *American Journal of Medical Genetics* **98**(3):235-243 DOI 10.1002/1096-8628(20010122)98:3<235.

Copela LA, Fernandez CF, Sherrer RL, Wolin SL. 2008. Competition between the Rex1 exonuclease and the La protein affects both Trf4p-mediated RNA quality control and pre-tRNA maturation. *RNA* **14**(6):1214–1227 DOI 10.1261/rna.1050408.

Crimi M, Sciacco M, Galbiati S, Bordoni A, Malferri G, Bo RDel, Biunno I, Bresolin N, Comi GP. 2002. A collection of 33 novel human mtDNA homoplasmic variants. *Human Mutation* **20**(5):409 DOI 10.1002/humu.9079.

Ding Y, Li Y, You J, Yang L, Chen B, Lu J, Guan MX. 2009. Mitochondrial tRNA(Glu) A14693G variant may modulate the phenotypic manifestation of deafness-associated 12S rRNA A1555G mutation in a Han Chinese family. *Journal of Genetics and Genomics* **36**(4):241–250 DOI 10.1016/S1673-8527(08)60111-3.

Ding Y, Teng YS, Zhuo GC, Xia BH, Leng JH. 2019. The mitochondrial tRNAHis G12192A mutation may modulate the clinical expression of deafness-associated tRNAThr G15927A mutation in a Chinese pedigree. *Current Molecular Medicine* **19**(2):136–146 DOI 10.2174/1566524019666190308121552.

Ding Y, Xia BH, Zhang CJ, Zhuo GC. 2017. Mutations in mitochondrial tRNA genes may be related to insulin resistance in women with polycystic ovary syndrome. *American Journal of Translational Research* **9**(6):2984–2996.

Ding Y, Xia BH, Zhang CJ, Zhuo GC. 2018. Mitochondrial tRNALeu(UUR) C3275T, tRNAGln T4363C and tRNALys A8343G mutations may be associated with PCOS and metabolic syndrome. *Gene* **642**:299–306 DOI 10.1016/j.gene.2017.11.049.

Ding Y, Ye YF, Li MY, Xia BH, Leng JH. 2020. Mitochondrial tRNAAla 5601C>T variant may affect the clinical expression of the LHONrelated ND4 11778G>A mutation in a family. *Molecular Medicine Reports* **21**(1):201–208 DOI 10.3892/mmr.2019.10844.

Duchen MR. 2004. Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Molecular Aspects of Medicine* **25**(4):365–451 DOI 10.1016/j.mam.2004.03.001.

Elson JL, Samuels DC, Turnbull DM, Chinnery PF. 2001. Random intracellular drift explains the clonal expansion of mitochondrial DNA mutations with age. *American Journal of Human Genetics* **68**(3):802–806 DOI 10.1086/318801.

El Yacoubi B, Bailly M, De Crécy-Lagard V. 2012. Biosynthesis and function of post-transcriptional modifications of transfer RNAs. *Annual Review of Genetics* **46**:69–95 DOI 10.1146/annurev-genet-110711-155641.

Finsterer J, Zarrouk-Majhoub S. 2018. Penetration of the LHON mutation m.11778G>A may depend on factors other than the haplotype or heteroplasmy rate. *Investigative Ophthalmology and Visual Science* **59**(1):381 DOI 10.1167/iovs.17-22983.
Florentz C, Sohm B, Tryoen-Tóth P, Pütz J, Sissler M. 2003. Human mitochondrial tRNAs in health and disease. *Cellular and Molecular Life Sciences* 60(7):1356–1375 DOI 10.1007/s00018-003-2343-1.

Garcia-Lozano JR, Aguilera I, Bautista J, Nunez-Roldan A. 2000. A new mitochondrial DNA mutation in the tRNA leucine 1 gene (C3275A) in a patient with Leber’s hereditary optic neuropathy. *Human Mutation* 15(1):120–121 DOI 10.1002/(SICI)1098-1004(200001)15:1<120::AID-HUMU33>3.0.CO;2-8.

Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. *Molecular Biology and Evolution* 30(5):1229–1235 DOI 10.1093/molbev/mst012.

Hanada T, Suzuki T, Yokogawa T, Takemoto-Hori C, Sprinzl M, Watanabe K. 2001. Translation ability of mitochondrial tRNAser with unusual secondary structures in an in vitro translation system of bovine mitochondria. *Genes to Cells* 6(12):1019–1030 DOI 10.1046/j.1365-2443.2001.00491.

Hartmann RK, Gössringer M, Spáth B, Fischer S, Marchfelder A. 2009. The making of tRNAs and more - RNase P and tRNase Z. *Progress in Molecular Biology and Translational Science* 85:319–368 DOI 10.1016/S0079-6603(08)00808-8.

Helm M, Brulé H, Friede D, Giegé R, Pütz D, Florentz C. 2000. Search for characteristic structural features of mammalian mitochondrial tRNAs. *RNA* 6(10):1356–1379 DOI 10.1017/s1355838200001047.

Holzmann J, Frank P, Löffler E, Bennett KL, Gerner C, Rossmanith W. 2008. RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. *Cell* 135(3):462–474 DOI 10.1016/j.cell.2008.09.013.

Howell N, Kubacka I, Xu M, McCullough DA. 1991. Leber hereditary optic neuropathy: involvement of the mitochondrial ND1 gene and evidence for an intragenic suppression mutation. *The American Journal of Human Genetics* 48(5):935–942.

Huoponen K, Vilkki J, Aula P, Nikoskelainen EK, Savontaus ML. 1991. A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. *American Journal of Human Genetics* 48(6):1147–1153 DOI 10.1016/0378-1119(91)90556-Q.

Ishii R, Minagawa A, Takaku H, Takagi M, Nashimoto M, Yokoyama S. 2005. Crystal structure of the tRNA 3′ processing endoribonuclease tRNase Z from Thermotoga maritima. *Journal of Biological Chemistry* 280(14):14138–14144 DOI 10.1074/jbc.M500355200.

Iwata-Reuyl D. 2008. An embarrassment of riches: the enzymology of RNA modification. *Current Opinion in Chemical Biology* 12(2):126–133 DOI 10.1016/j.cbpa.2008.01.041.

Jancic J, Rovcanin B, Djuric V, Pepic A, Samardzic J, Nikolic B, Novakovic I, Kostic VS. 2020. Analysis of secondary mtDNA mutations in families with Leber’s hereditary optic neuropathy: four novel variants and their association with clinical presentation. *Mitochondrion* 50:132–138 DOI 10.1016/j.mito.2019.10.011.

Jia X, Li S, Xiao X, Guo X, Zhang Q. 2006. Molecular epidemiology of mtDNA mutations in 903 Chinese families suspected with Leber hereditary optic neuropathy. *Journal of Human Genetics* 51(10):851–856 DOI 10.1007/s10038-006-0032-2.

Jiang P, Jin X, Peng Y, Wang M, Liu H, Liu X, Zhang Z, Ji Y, Zhang J, Liang M, Zhao F, Sun YH, Zhang M, Zhou X, Chen Y, Mo JQ, Huang T, Qu J, Guan MX. 2016. The
exome sequencing identified the mutation in YARS2 encoding the mitochondrial
 tyrosyl-tRNA synthetase as a nuclear modifier for the phenotypic manifestation
 of Leber’s hereditary optic neuropathy-associated mitochondrial DNA mutation.  
*Human Molecular Genetics* **25**(3):584–596 DOI 10.1093/hmg/ddv498.

Ji Y, Qiao L, Liang X, Zhu L, Gao Y, Zhang J, Jia Z, Wei QP, Liu X, Jiang P, Guan 
MX. 2017. Leber’s hereditary optic neuropathy is potentially associated with 
 a novel m.5587T>C mutation in two pedigrees. *Molecular Medicine Reports*  
**16**(6):8997–9004 DOI 10.3892/mmr.2017.7734.

Ji Y, Zhang AM, Jia X, Zhang YP, Xiao X, Li S, Guo X, Bandelt HJ, Zhang Q, Yao 
YG. 2008. Mitochondrial DNA haplogroups M7b1/2 and M8a affect clinical 
expression of leber hereditary optic neuropathy in Chinese families with the
 m.11778G→a mutation. *American Journal of Human Genetics* **83**(6):760–768 
DOI 10.1016/j.ajhg.2008.11.002.

Jia Z, Wang X, Qin Y, Xue L, Jiang P, Meng Y, Shi S, Wang Y, Mo JQin, Guan MX. 2013.  
Coronary heart disease is associated with a mutation in mitochondrial tRNA. *Human 
Molecular Genetics* **22**(20):4064–4073 DOI 10.1093/hmg/ddt256.

Jia Z, Zhang Y, Li Q, Ye Z, Liu Y, Fu C, Cang X, Wang M, Guan MX. 2019. A 
coronary artery disease-associated tRNAThr mutation altered mitochondrial 
function, apoptosis and angiogenesis. *Nucleic Acids Research* **47**(4):2056–2074 
DOI 10.1093/nar/gky1241.

Johns DR, Neufeld MJ, Park RD. 1992. An ND-6 mitochondrial DNA mutation 
associated with Leber hereditary optic neuropathy. *Biochemical and Biophysical 
Research Communications* **187**(3):1551–1557 DOI 10.1016/0006-291x(92)90479-5.

Kirchner S, Ignatova Z. 2015. Emerging roles of tRNA in adaptive translation, signaling 
dynamics and disease. *Nature Reviews Genetics* **16**(2):98–112 DOI 10.1038/nrg3861.

Lane N, Martin W. 2010. The energetics of genome complexity. *Nature* **467**(7318):929–934 
DOI 10.1038/nature09486.

Leigh-Brown S, Enriquez JA, Odom DT. 2010. Nuclear transcription factors in mamma-
lian mitochondrial. *Genome Biology* **11**(7):215 DOI 10.1186/gb-2010-11-7-215.

Li R, Qu J, Zhou X, Tong Y, Hu Y, Qian Y, Lu F, Mo JQ, West CE, Guan MX. 2006.  
The mitochondrial tRNA(Thr) A15951G mutation may influence the phenotypic 
expression of the LHON-associated ND4 G11778A mutation in a Chinese family.  
*Gene* **376**(1):79–86 DOI 10.1016/j.gene.2006.02.014.

Li S, Duan S, Qin Y, Lin S, Zheng K, Li X, Zhang L, Gu X, Yao K, Wang B. 2019. Leber’s 
Hereditary Optic Neuropathy-specific heteroplasmic mutation m.14495A>G 
found in a Chinese family. *Translational Vision Science & Technology* **8**(4):3 
DOI 10.1167/tvst.8.4.3.

Li de la Sierra-Gallay I, Pellegrini O, Condon C. 2005. Structural basis for sub-
strate binding, cleavage and allostery in the tRNA maturase RNase Z. *Nature*  
**433**(7026):657–661 DOI 10.1038/nature03284.

Ling J, Reynolds N, Ibba M. 2009. Aminoacyl-tRNA synthesis and translational quality 
control. *Annual Review of Microbiology* **63**:61–78 
DOI 10.1146/annurev.micro.091208.073210.
Liu Y, Li R, Li Z, Wang XJ, Yang L, Wang S, Guan MX. 2009. Mitochondrial transfer tRNAMet 4435A>G mutation is associated with maternally inherited hypertension in a Chinese pedigree. *Hypertension* **53**(6):1083–1090 DOI 10.1161/HYPERTENSIONAHA.109.128702.

Lu Z, Chen H, Meng Y, Wang Y, Xue L, Zhi S, Qiu Q, Yang L, Mo JQ, Guan MX. 2011. The tRNAMet 4435A>G mutation in the mitochondrial haplogroup G2a1 is responsible for maternally inherited hypertension in a Chinese pedigree. *European Journal of Human Genetics* **19**(11):1181–1186 DOI 10.1038/ejhg.2011.111.

Luo S, Valencia CA, Zhang J, Lee NC, Slone J, Gui B, Wang X, Li Z, Dell S, Brown J, Chen SM, Chien YH, Hwu WL, Fan PC, Wong LJ, Atwal PS, Huang T. 2018. Biparental inheritance of mitochondrial DNA in humans. *Proceedings of The National Academy of Sciences of The United States of America* **115**(51):13039–13044 DOI 10.1073/pnas.1810946115.

Lyu Y, Xu M, Chen J, Ji Y, Guan MX, Zhang J. 2019. Frequency and spectrum of MT-IT variants associated with Leber’s hereditary optic neuropathy in a Chinese cohort of subjects. *Mitochondrial DNA Part B Resources* **4**(2):2266–2280 DOI 10.1080/23802359.2019.1627921.

Mackey D, Howell N. 1992. A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. *American Journal of Human Genetics* **51**(6):1218–1228.

Man PY, Turnbull DM, Chinnery PF. 2002. Leber hereditary optic neuropathy. *Journal of Medical Genetics* **39**(3):162–169 DOI 10.1136/jmg.39.3.162.

Man X, CI X, Lyu Y, Zhang J, Guan M. 2020. The mitochondrial tRNAPhe 593T>C mutation may influence the Phenotypic expression of the LHON associated m.1448T>C mutation. *Journal of Wenzhou Medical University* **50**(5):356–363.

Maraia RJ, Lamichhane TN. 2011. 3′ processing of eukaryotic precursor tRNAs. *Wiley Interdisciplinary Reviews-RNA* **2**(3):362–375 DOI 10.1002/wrna.64.

Mimaki M, Ikota A, Sato A, Komaki H, Akanuma J, Nonaka I, Goto Y. 2003. A double mutation (G11778A and G12192A) in mitochondrial DNA associated with Leber’s hereditary optic neuropathy and cardiomyopathy. *Journal of Human Genetics* **48**(1):47–50 DOI 10.1007/s100380300005.

Nagao A, Suzuki T, Katoh T, Sakaguchi Y, Suzuki T. 2009. Biogenesis of glutaminyl-mt tRNAGln in human mitochondria. *Proceedings of The National Academy of Sciences of The United States of America* **106**(38):16209–16214 DOI 10.1073/pnas.0907602106.

Neckelmann N, Li K, Wade RP, Shuster R, Wallace DC. 1987. cDNA sequence of a human skeletal muscle ADP/ATP translocator: lack of a leader peptide, divergence from a fibroblast translocator cDNA, and coevolution with mitochondrial DNA genes. *Proceedings of The National Academy of Sciences of The United States of America* **84**(21):7580–7584 DOI 10.1073/pnas.84.21.7580.

Newman NJ, Lott MT, Wallace DC. 1991. The clinical characteristics of pedigrees of Leber’s hereditary optic neuropathy with the 11778 mutation. *American Journal of Ophthalmology* **111**(6):750–762 DOI 10.1016/s0002-9394(14)76784-4.
Ozanick SG, Wang X, Costanzo M, Brost RL, Boone C, Anderson JT. 2009. Rex1p deficiency leads to accumulation of precursor initiator tRNAMet and polyadenylation of substrate RNAs in Saccharomyces cerevisiae. Nucleic Acids Research 37(1):298–308 DOI 10.1093/nar/gkn925.

Paris Z, Alfonzo JD. 2018. How the intracellular partitioning of tRNA and tRNA modification enzymes affects mitochondrial function. IUBMB Life 70(12):1207–1213 DOI 10.1002/iub.1957.

Phizicky EM, Hopper AK. 2010. tRNA biology charges to the front. Genes & Development 24(17):1832–1860 DOI 10.1101/gad.1956510.

Pütz J, Dupuis B, Sissler M, Florentz C. 2007. Mamit-tRNA, a database of mammalian mitochondrial tRNA primary and secondary structures. RNA 13(8):1184–1190 DOI 10.1261/rna.588407.

Qu J, Li R, Zhou X, Tong Y, Lu F, Qian Y, Hu Y, Mo JQ, West CE, Guan MX. 2006. The novel A4435G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. Investigative Ophthalmology & Visual Science 47(2):475–483 DOI 10.1167/iovs.05-0665.

Rizzuto R, De Stefani D, Raffaello A, Mammucari C. 2012. Mitochondria as sensors and regulators of calcium signalling. Nature Reviews Molecular Cell Biology 13(9):566–578 DOI 10.1038/nrm3412.

Rosenberg T, Norby S, Schwartz M, Saillard J, Magalhães PJ, Leroy D, Kann EC, Duno M. 2016. Prevalence and genetics of Leber hereditary optic neuropathy in the Danish population. Investigative Ophthalmology & Visual Science 57(3):1370–1375 DOI 10.1167/iovs.15-18306.

Rossmanith W. 1997. Processing of human mitochondrial tRNAser(AGY): a novel pathway in tRNA biosynthesis. Journal of Molecular Biology 265(4):365–371 DOI 10.1006/jmbi.1996.0750.

Rossmanith W, Tullo A, Potuschak T, Karwan R, Sbisà E. 1995. Human mitochondrial tRNA processing. Journal of Biological Chemistry 270(21):12885–12891 DOI 10.1074/jbc.270.21.12885.

Salinas-Giegé T, Giegé R, Giegé P. 2015. tRNA biology in mitochondria. International Journal of Molecular Sciences 16(3):4518–4559 DOI 10.3390/ijms16034518.

Sandbach JM, Newman NJ. 2001. Retinal masqueraders of optic nerve disease. Ophthalmology Clinics of North America 14(1):41–59.

Scaglia F, Wong LJ. 2008. Human mitochondrial transfer RNAs: role of pathogenic mutation in disease. Muscle & Nerve 37(2):150–171 DOI 10.1002/mus.20917.

Sena LA, Chandel NS. 2012. Physiological roles of mitochondrial reactive oxygen species. Molecular Cell 48(2):158–167 DOI 10.1016/j.molcel.2012.09.025.

Stowe DF, Camara AK. 2009. Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. Antioxidants & Redox Signaling 11(6):1373–1414 DOI 10.1089/ars.2008.2331.

Suzuki T, Nagao A, Suzuki T. 2011. Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. Annual Review of Genetics 45:299–329 DOI 10.1146/annurev-genet-110410-132531.
Suzuki T, Suzuki T, Wada T, Saigo K, Watanabe K. 2002. Taurine as a constituent of mitochondrial tRNAs: new insights into the functions of taurine and human mitochondrial diseases. *The EMBO Journal* 21(23):6581–6589 DOI 10.1093/emboj/cdf656.

Taanman JW. 1999. The mitochondrial genome: structure, transcription, translation and replication. *Biochimica Et Biophysica Acta* 1410(2):103–123 DOI 10.1016/s0005-2728(98)00161-3.

Tait SW, Green DR. 2010. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nature Reviews Molecular Cell Biology* 11(9):621–632 DOI 10.1038/nrm2952.

Tang DL, Zhou X, Li X, Zhao L, Liu F. 2006. Variation of mitochondrial gene and the association with type 2 diabetes mellitus in a Chinese population. *Diabetes Research and Clinical Practice* 73(1):77–82 DOI 10.1016/j.diabres.2005.12.001.

Tong Y, Mao Y, Zhou X, Yang L, Zhang J, Cai W, Zhao F, Wang X, Lu F, Qu J, Guan MX. 2007. The mitochondrial tRNA(Glu) A14693G mutation may influence the phenotypic manifestation of ND1 G3460A mutation in a Chinese family with Leber’s hereditary optic neuropathy. *Biochemical and Biophysical Research Communications* 357(2):524–530 DOI 10.1016/j.bbrc.2007.03.189.

Tońska K, Kodroń A, Bartnik E. 2010. Genotype-phenotype correlations in Leber hereditary optic neuropathy. *Biochimica et Biophysica Acta* 1797(6-7):1119–1123 DOI 10.1016/j.bbabio.2010.02.032.

Tzen CY, Thajeb P, Wu TY, Chen SC. 2003. Melas with point mutations involving tRNALeu(A3243G) and tRNAGlu(A14693g). *Muscle & Nerve* 28(5):575–581 DOI 10.1002/mus.10473.

Ueda K, Morizane Y, Shiraga F, Shikishima K, Ishikawa H, Wakakura M, Nakamura M. 2017. Nationwide epidemiological survey of Leber hereditary optic neuropathy in Japan. *Journal of Epidemiology* 27(9):447–450 DOI 10.1016/j.jje.2017.02.001.

Ueda T, Yotsumoto Y, Ikeda K, Watanabe K. 1992. The T-loop region of animal mitochondrial tRNA(Ser)(AGY) is a main recognition site for homologous seryl-tRNA synthetase. *Nucleic Acids Research* 20(9):2217–2222 DOI 10.1093/nar/20.9.2217.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, 2nd Elsas LJ, Nikoskelainen EK. 1988. Mitochondrial DNA mutation associated with Leber’s hereditary optic neuropathy. *Science* 242(4884):1427–1430 DOI 10.1126/science.3201231.
Wang X, Lu J, Zhu Y, Yang A, Yang L, Li R, Chen B, Qian Y, Tang X, Wang J, Zhang X, Guan MX. 2008. Mitochondrial tRNAThr G15927A mutation may modulate the phenotypic manifestation of ototoxic 12S rRNA A1555G mutation in four Chinese families. *Pharmacogenetics and Genomics* **18**(12):1059–1070 DOI 10.1097/FPC.0b013e3283131661.

Watanabe Y, Kawai G, Yokogawa T, Hayashi N, Kumazawa Y, Ueda T, Nishikawa K, Hirao I, Miura K, Watanabe K. 1994. Higher-order structure of bovine mitochondrial tRNAser(UGA): chemical modification and computer modeling. *Nucleic Acids Research* **22**(24):5378–5784 DOI 10.1093/nar/22.24.5378.

Yakes FM, Van Houten B. 1997. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proceedings of The National Academy of Sciences of The United States of America* **94**(2):514–519 DOI 10.1073/pnas.94.2.514.

Yao P, Fox PL. 2013. Aminoacyl-tRNA synthetases in medicine and disease. *EMBO Molecular Medicine* **5**(3):332–343 DOI 10.1002/emmm.201100626.

Yu J, Liang X, Ji Y, Ai C, Liu J, Zhu L, Nie Z, Jin X, Wang C, Zhang J, Zhao F, Mei S, Zhao X, Zhou X, Zhang M, Wang M, Huang T, Jiang P, Guan MX. 2020. PRICKLE3 linked to ATPase biogenesis manifested Leber’s hereditary optic neuropathy. *Journal of Clinical Investigation* **130**(9):4935–4946 DOI 10.1172/JCI134965.

Yu-Wai-Man P, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. 2003. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *American Journal of Human Genetics* **72**(2):333–339 DOI 10.1086/346066.

Yu-Wai-Man P, Griffiths PG, Chinnery PF. 2011. Mitochondrial optic neuropathies—disease mechanisms and therapeutic strategies. *Progress in Retinal and Eye Research* **30**(2):81–114 DOI 10.1016/j.preteyeres.2010.11.002.

Zhang AM, Bandelt HJ, Jia X, Zhang W, Li S, Yu D, Wang D, Zhuang XY, Zhang Q, Yao YG. 2011. Is mitochondrial tRNA(phe) variant m.593T>C a synergistically pathogenic mutation in Chinese LHON families with m.11778G>A?. *PLOS ONE* **6**(10):e26511 DOI 10.1371/journal.pone.0026511.

Zhang J, Ji Y, Liu X, Chen J, Wang B, Zhang M, Guan MX. 2018. Leber’s hereditary optic neuropathy caused by a mutation in mitochondrial tRNAThr in eight Chinese pedigrees. *Mitochondrion* **42**:84–91 DOI 10.1016/j.mito.2017.12.003.

Zhang YM, Ji YC, Liu XL, Zhou XT, Zhao FX, Sun YH, Wei QP, Zhang JJ, Liu Y, Qu J, Guan MX. 2010. Leber’s hereditary optic neuropathy may be associated with the mitochondrial tRNAGlu A14693G mutation in three Chinese families. *Yi Chuan* **32**(4):353–359 DOI 10.3724/sp.j.1005.2010.00353.

Zheng P, Li S, Liu C, Zha Z, Wei X, Yuan Y. 2018. Mitochondrial tRNA(Ala) C5601T mutation may modulate the clinical expression of tRNA(Met) A4435G mutation.
in a Han Chinese family with hypertension. *Clinical and Experimental Hypertension* **40**(6):595–600 DOI 10.1080/10641963.2017.1411497.

Zhou HH, Dai XN, Lin B, Mi H, Liu XL, Zhao FX, Zhang JJ, Zhou XT, Sun YH, Wei QP, Qu J, Guan MX. 2012. The analysis of Leber’s hereditary optic neuropathy associated with mitochondrial tRNAAla C5601T mutation in seven Han Chinese families. *Yi Chuan* **34**(8):1031–1042 DOI 10.3724/sp.j.1005.2012.01031.

Zhou M, Xue L, Chen Y, Li H, He Q, Wang B, Meng F, Wang M, Guan MX. 2018. A hypertension-associated mitochondrial DNA mutation introduces an M$^1$G37 modification into tRNAMetaltering its structure and function. *Journal of Biological Chemistry* **293**(4):1425–1438 DOI 10.1074/jbc.RA117.000317.