Abstract. Certain mutations in mitochondrial DNA (mtDNA) are associated with Leber's hereditary optic neuropathy (LHON). In particular, the well-known NADH dehydrogenase 4 (ND4) m.11778G>a mutation is one of the most common LHON-associated primary mutations worldwide. However, how specific mtDNA mutations, or variants, affect LHON penetrance is not fully understood. The aim of the current study was to explore the relationship between mtDNA mutations and LHON, and to provide useful information for early detection and prevention of this disease. Following the molecular characterization of a Han Chinese family with maternally inherited LHON, four out of eight matrilineal relatives demonstrated varying degrees of both visual impairment and age of onset. Through PCR amplification of mitochondrial genomes and direct Sanger sequencing analysis, a homoplasmic mitochondrial-encoded ND4 m.11778G>a mutation, alongside a set of genetic variations belonging to human mtDNA haplogroup B5b1 were identified. Among these sequence variants, alanine transfer tRNA (tRNA)ala m.5601c>T was of particular interest. This variant occurred at position 59 in the Tψc loop and altered the base pairing, which led to mitochondrial RNA (mtRNA) metabolism failure and defects in mitochondrial protein synthesis. Bioinformatics analysis suggested that the m.5601c>T variant altered tRNAala structure. Therefore, impaired mitochondrial functions caused by the ND4 m.11778G>A mutation may be enhanced by the mt-tRNAAla m.5601C>T variant. These findings suggested that the tRNAAla m.5601C>T variant might modulate the clinical manifestation of the LHON-associated primary mutation.

Introduction

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease that affects 1 in 31,000-50,000 people and culminates in the bilateral loss of central vision (1-3). In the North East of England, it has been reported that 1:8,500 individuals harbor a primary LHON-causing mutation and 1:31,000 experience visual loss as a result of LHON (4). Patients with LHON may exhibit abnormal symptoms, including movement disorders, dystonia or multiple sclerosis-like symptoms, which pose a significant challenge for clinicians (5,6). Few significant improvements in visual acuity are reported following atrophy of the optic discs. LHON demonstrates an incomplete penetrance for both vision loss and gender bias; LHON affects males more frequently than females (7,8). Three primary mutations including the NADH dehydrogenase (ND) 4 m.11778G>A, ND6 m.14484T>C and ND1 m.3460G>A have been identified in 90% of patients with LHON (9-11). Yet the molecular mechanisms of these mtDNA mutations in the phenotypic manifestation of LHON have not been elucidated.

To understand the role of mitochondrial dysfunction in LHON, an extended genetic screen for mtDNA variants was performed in a Han Chinese family with a high prevalence of LHON. Sequence analysis of the complete mitochondrial genome identified the occurrence of an ND4 m.11778G>A mutation and an alanine transfer RNA (tRNAAla) m.5601C>T variant within matrilineal relatives of the proband. In addition, bioinformatics analysis was performed in order to explore whether the m.5601C>T variant affected the tRNAAla secondary structure.

Patients and methods

Patients and genetic screening. To identify mtDNA variations in Chinese patients with LHON, a Han Chinese family was recruited from Hangzhou First People's Hospital (Hangzhou, China) in January 2018, and blood samples (5 ml) were collected from each matrilineal relative of the proband. Blood samples (5 ml) from unrelated control subjects (n=300) from the same geographical region recruited at the Hangzhou First People's Hospital were also used in the present study. These
healthy subjects consisted of 200 males and 100 females, aged 11-48 years, and were enrolled from January 2018 to January 2019. The present study was approved by the Ethics Committee of Hangzhou First People's Hospital. Written informed consent was obtained from all participants, or their parent/guardian, prior to enrollment in the study.

Clinical examinations. The proband (II-12) and other affected matrilineal relatives (II-5, II-7 and III-8; Fig. 1) underwent comprehensive ophthalmic examinations, including visual field tests, examination of visual acuity, fundus photography, visual evoked potentials and determination of the degree of visual impairment, performed as previously described (12,13). The degree of visual impairment was classified based on the following criteria (12,13): healthy, ≥0.300; mild, 0.100-0.299; moderate, 0.050-0.099; severe, 0.020-0.049; and profound, <0.020.

PCR and genetic sequencing to identify mtDNA variants. Genomic DNA from LHON patients and control subjects was extracted using a DNA extraction kit (Qiagen® DNA Blood Mini kit; Qiagen GmbH), according to the manufacturer's protocol. The complete mitochondrial genomes of II-5, II-7, II-12 and III-8 were amplified in 24 overlapping fragments using 200 µM dNTP, 10X buffer, Taq DNA polymerase and 15 mmol/l Mg²⁺ (cat. no. R004A; Takara Biotechnology, Co., Ltd.). The 24 sequences of light-strand and heavy-strands oligonucleotide primers for amplification of mtDNA genes were used according to a previous report (14). The following thermocycling conditions were used for PCR: 95°C for 5 min; 30 cycles of 94°C for 10 sec, 60°C for 30 sec and 72°C for 1 min; and a final extension at 72°C for 5 min. After confirmation of band of interest, the PCR products were purified using the PureLink Gel Extraction kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's recommendations. DNA samples with concentrations >1.0 ng/µl were sequenced using the BigDye™ Terminator Cycle Sequencing reaction kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and an ABI PRISM® 3700 DNA Analyzer. Sequencing data were compared with the updated Xenopus laevis presented in Table I. Mitochondrial DNA sequence accession number of 17 vertebrate species used in the phylogenetic analyses.

| Species               | GenBank accession no. |
|-----------------------|------------------------|
| Homo sapiens          | NC_012920              |
| Cebus albifrons       | NC_002763              |
| Gorilla gorilla       | NC_011120              |
| Hyllobates lar        | NC_002082              |
| Lemur catta           | NC_004025              |
| Macaca mulatta        | NC_005943              |
| Macaca sylvanus       | NC_002764              |
| Nycticebus coucang    | NC_002765              |
| Pan paniscus          | NC_001644              |
| Pan troglodytes       | NC_001643              |
| Papio hamadryas       | NC_001992              |
| Pongo pygmaeus        | NC_001646              |
| Pongo pygmaeus abelii | NC_002083              |
| Tarsius bancanus      | NC_002811              |
| Mus musculus          | NC_006914.1            |
| Bos taurus            | HM045018.1             |
| Xenopus laevis        | NC_001573.1            |

Phylogenetic conservation analysis. Phylogenetic analysis was performed to determine the potential pathogenic role of the identified mtDNA mutations. Briefly, 17 different species were selected for phylogenetic analysis (Table I). The conservation index (CI) was measured by comparing the human nucleotide alternations with the nucleotide sequences of other species. CI≥70% was implicated to have functional significance (16).

Bioinformatics analysis. To determine whether the m.5601C>T variant affected tRNA^{Aua} secondary structure, the RNAfold web server program was used (http://rna.bii.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi), as previously described (17).

Determining the pathogenicity of the variant. The role of the tRNA^{Aua} m.5601C>T variant was determined using the pathogenicity scoring system, as described by Yarham et al (18). In brief, mutations were classified as: ‘neutral polymorphism’, ≤6; ‘possible pathogenic’, 7-10; ‘definitely pathogenic’, ≥11.

Statistical analysis. SPSS 17.0 software (SPSS Inc.) was used for statistical analysis. Fisher's exact test was used to assess the differences between groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical presentation of a Han Chinese family with LHON. A pedigree chart from a Han Chinese family with a history of LHON is presented in Fig. 1. There were four LHON patients presented in the pedigree (three males and one female), aged 7-39 years old. Medical history analysis of the proband (II-12) confirmed that no other clinical disorders, such as deafness, diabetes mellitus, cardiovascular diseases, cancer or neurological disorders, were present. Following comprehensive genetic counseling at the Department of Ophthalmology in Hangzhou First People's Hospital, the proband (age, 39), was found to have begun suffering from painless and progressive bilateral loss of vision at the age of 19, manifesting as a dark cloud in the central vision and difficulty differentiating different colors. Ophthalmic examination revealed large centrocecal scotoma in both eyes, a typical clinical feature of LHON (13). A total of three out of seven matrilineal relatives (II-5, II-7 and III-8), in addition to the proband (II-12), suffered from moderate to profound visual impairment (Table II).

Screening for mtDNA mutations. To investigate the molecular basis of LHON, II-5, II-7, II-12 and III-8 were screened for mutations following PCR amplification of the mtDNA genomes. Sequence analysis of the mtDNA PCR products
revealed 32 genetic polymorphisms (Table III), all of which belonged to the human mtDNA B5b1 haplogroup (19). Of these, there were nine variants in the D-loop gene, two variants in the 12S rRNA gene, one variant in the 16S rRNA gene and one variant in a tRNA gene (m.5601C>T). The other variants were mainly localized within oxidative phosphorylation encoding genes. Notably, five missense mutations were identified: mitochondrial encoded NADH dehydrogenase 1 (ND1) m.3593T>C (p.V96A), ND2 m.5442T>C (p.F193L), ATP 6 m.9103T>C (p.F325L), ND3 m.10398A>G (p.T114A) and ND4 m.11778G>A (p.R340H). The cis of these variants were investigated between different species, including mouse, bovine and *Xenopus laevis* (20-22). Of all identified variants, only tRNA^Ala^ m.5601C>T and ND4 m.11778G>A were conserved. Notably, the m.5601C>T and m.11778G>A mutations were absent in the 300 control subjects compared with the mtDNA genomes of the matrilineal relatives (P<0.05). Taken together, these results indicated that tRNA^Ala^ m.5601C>T and ND4 m.11778G>A may have active roles in the pathogenesis of LHON.

In addition, the results revealed that the ratio between affected males and females carrying ND4 m.11778G>A mutations in this case study was 3:1, which was similar to previous studies on families with LHON carrying ND4 m.11778G>A mutations (Table IV) (23-28). These findings suggested that the m.11778G>A mutation may be the molecular basis for the LHON phenotype.

**m.5601C>T variant alters the tRNA^Ala^ structure.** The m.5601C>T variant is located at a highly conserved position in the TψC loop within the tRNA^Ala^ (29); thus, the point mutation results in a missense mutation that creates a novel base pairing (55T-59C) (Figs. 2 and 3). Subsequent bioinformatics analysis revealed that the m.5601C>T variant caused a structural alteration of tRNA^Ala^ (Fig. 4), which indicated that m.5601C>T may have an impact on tRNA^Ala^ function.

**m.5601C>T variant is ‘possibly pathogenic’ for LHON.** The pathogenicity scoring system described by Yarham et al (18) was used to determine the role of the tRNA^Ala^ m.5601C>T variant. As presented in Table V, the total pathogenicity score for the m.5601C>T variant was 8, placing it within the ‘possibly pathogenic’ category for LHON.

**Discussion**

In the present study, a Han Chinese family with maternally inherited LHON was clinically and molecularly characterized. One of the most common features of LHON is bilateral loss of vision in the matrilineal relatives of the proband (9); this preferential effect on vision has facilitated the positive association between mtDNA mutations and LHON (30). Clinical evaluation of this family revealed that the age of onset for visual impairment between 3 and 19 years. The association between m.11778G>A and LHON was reported as early as 1988 (31). In the present study, patients harboring the m.11778G>A mutation had different mtDNA haplogroups, suggesting that the m.11778G>A mutation occurred sporadically and multiplied through evolution of the mtDNA in China. The varying degree of visual impairment in this Chinese family suggested that modifying factors, such as nuclear genes, environmental factors and mitochondrial genetic polymorphisms, may also contribute to LHON penetrance (32). In particular, secondary LHON-associated variants, such as ND1 m.4216T>C and ND5 m.13708G>A mutations in the mtDNA haplogroup J, may increase the penetrance and severity of LHON, in combination with the primary mutations, in European populations (33). mtDNA haplogroups M7b1’2 and M8a have been implicated in the clinical expression of the LHON-associated ND4 m.11778G>A mutation (33).

In the present study, sequencing of the complete mitochondrial genomes of the matrilineal relatives (II-5, II-7, II-12 and III-8) revealed a set of genetic polymorphisms from the Asian mtDNA haplogroup B5b1 (19). Of these variants, tRNA^Ala^ m.5601C>T was of most interest because this variant is located at a highly conserved nucleotide in the TψC loop of tRNA^Ala^ (position 59), which is thought to be involved in tertiary interactions between the TψC loop and the truncated D-arm (34). Bioinformatics analysis revealed that the m.5601C>T variant created a novel Watson-Crick base-pairing (55T-59C). The tRNA^Ala^ m.5601C>T variant has previously been associated with maternally inherited hypertension and mitochondrial...
Table II. Summary of the clinical data for the proband (II-12) and matrilineal relatives (II-5, II-7 and III-8) in the Han Chinese family with maternally inherited Leber's hereditary optic neuropathy.

| Subject | Sex  | Age at onset (years) | Age at test (years) | Visual impairment score | Degree of visual impairment |
|---------|------|----------------------|---------------------|-------------------------|-----------------------------|
| II-5    | Male | 11                   | 35                  | 0.03                    | Severe                      |
| II-7    | Male | 16                   | 33                  | 0.1                     | Moderate                    |
| II-12   | Female | 19                  | 39                  | 0.01                    | Profound                    |
| III-8   | Male | 3                    | 7                   | 0.02                    | Profound                    |

*The degree of visual impairment was classified based on criteria stated in the clinical examinations section of the Methods.

Table III. Sequence analysis of mitochondrial DNA mutations in a Han Chinese family with maternally inherited Leber's hereditary optic neuropathy.

| Gene | Position | Base change | Conservation (H/B/M/X)* | CI (%) | Previously reportedb |
|------|----------|-------------|-------------------------|--------|-----------------------|
| D-loop | 73 | A>G     |                         |        | Yes                   |
|       | 152      | T>C       |                         |        | Yes                   |
|       | 189      | A>C       |                         |        | Yes                   |
|       | 263      | A>G       |                         |        | Yes                   |
|       | 489      | T>C       |                         |        | Yes                   |
|       | 16117    | T>C       |                         |        | Yes                   |
|       | 16172    | T>C       |                         |        | Yes                   |
|       | 16223    | T>C       |                         |        | Yes                   |
|       | 16519    | T>C       |                         |        | Yes                   |
| 12S rRNA | 709 | G>A     |                         |        | Yes                   |
|       | 1438     | A>G       |                         |        | Yes                   |
| 16S rRNA | 2706 | A>G     |                         |        | Yes                   |
| ND1   | 3593     | T>C (p.V96A) | V/I/I/A | 25     | Yes                   |
|       | 4102     |           |                         |        | Yes                   |
| ND2   | 4769     | A>G       |                         |        | Yes                   |
|       | 4833     | A>G       |                         |        | Yes                   |
|       | 5108     | T>C       |                         |        | Yes                   |
|       | 5442     | T>C (p. F325L) | F/F/M/L | 23     | Yes                   |
| tRNAAla | 5601 | C>T     |                         |        | Yes                   |
| CO1   | 7028     | C>T       |                         |        | Yes                   |
|       | 7600     | G>A       |                         |        | Yes                   |
| CO2   | 8167     | C>T       |                         |        | Yes                   |
| ATP6  | 8547     | C>T       |                         |        | Yes                   |
|       | 8748     | C>T       |                         |        | Yes                   |
|       | 9103     | T>C (p. F193L) | F/F/F/S | 52     | Yes                   |
| ND3   | 10398    | A>G (p. T114A) | T/T/T/A | 36     | Yes                   |
| ND4   | 11719    | G>A       |                         |        | Yes                   |
|       | 11778    | G>A (p. R340H) | R/R/R/R | 100    | Yes                   |
| ND5   | 12705    | C>T       |                         |        | Yes                   |
| ND6   | 14668    | C>T       |                         |        | Yes                   |
| Cyt b | 15043    | G>A       |                         |        | Yes                   |
|       | 15301    | G>A       |                         |        | Yes                   |

*Conservation of amino acid for polypeptide. bFrom Mitomap database (www.mitomap.org). Conserved nucleotide residues are shown in bold font. B, bovine; CO, cytochrome c oxidase; cyt b, cytochrome b; H, human; M, mouse; ND, mitochondrial encoded NADH dehydrogenase; rRNA, ribosomal RNA; tRNA, transfer RNA; X, Xenopus laevis.
myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (35,36). Therefore, the m.5601 c>T may alter the secondary structure of tRNA<sup>ala</sup> and impair the mt-tRNA metabolism and protein translation, and contribute to the LHON phenotype. A previous study has demonstrated that the m.12192G>a mutation, occurring at a similar position on tRNA<sup>His</sup>, modulates the clinical expression of deafness in a Chinese pedigree (37), whereas the m.5601 c>T variant may increase the penetrance of the hypertension-associated tRNA<sup>Met</sup> m.4435a>G mutation (35). Zhou et al have previously described the association between the tRNA<sup>ala</sup> m.5601c>T variant and LHON in seven Han Chinese families (38). However, these families only carried the m.5601c>T variant, and did not harbor the three LHON-associated primary mutations (ND4 m.11778G>a, ND6 m.14484T>c and ND1 m.3460G>a), thus exhibiting very low penetrance and severity of visual impairment (4.5-25.0%) (38). In the present study, the penetrance of LHON-induced visual impairment was 40%, which suggested that the combination of the ND4 m.11778G>A mutation and the tRNA<sup>ala</sup> m.5601C>T variant may be responsible for the higher prevalence of LHON in this family.

Results from the present study suggested that the tRNA<sup>ala</sup> m.5601C>T variant could increase both the prevalence and

Table IV. Clinical and molecular data for eight Han Chinese pedigrees carrying the ND4 11778G>A primary mutation in LHON.

| Author, year      | Pedigree number | Affected ratio (male:female) | Penetrance of LHON (%) | Secondary variants | MitDNA haplogroup | (Refs.) |
|-------------------|-----------------|------------------------------|------------------------|--------------------|-------------------|--------|
| Ding et al, 2019  | 1               | 3:1                          | 40                     | tRNA<sup>ala</sup> m.5601C>T | B5b1              | -      |
| Qu et al, 2006    | 2               | 3:1                          | 61.5                   | tRNA<sup>met</sup> m.4435A>G | D5                | (23)   |
| Li et al, 2006    | 3               | 2:1                          | 60                     | tRNA<sup>Met</sup> m.15951A>G | D4                | (24)   |
| Zhang et al, 2010 | 4               | 3:0                          | 37.5                   | ND1 m.3394T>C      | M9a               | (25)   |
| Qu et al, 2007    | 5               | 3.5:1                        | 33                     | ND4 m.11696G>C     | D4                | (26)   |
| Zhang et al, 2010 | 6               | 1:1                          | 57.1                   | ND6 m.14502T>C     | M10a              | (27)   |
| Qu et al, 2009    | 7               | 1:0                          | 14.2                   | None               | M8a2              | (28)   |
| Qu et al, 2009    | 8               | 2:0                          | 8                      | None               | D4g2              | (28)   |

LHON, Leber’s hereditary optic neuropathy; ND, mitochondrial encoded NADH dehydrogenase; tRNA, transfer RNA.
the expression of the LHON-associated ND4 m.11778G>A mutation. Evidence to support this includes the fact that the mutation occurs at a highly conserved nucleotide of tRNA\(^{\text{Ala}}\), which is critical for basal tRNA activity and normal function (29). The present data demonstrated that the m.5601C>T variant alters the secondary structure of the tRNA\(^{\text{Ala}}\) gene. Finally, the pathogenicity scoring system generated indicated that the m.5601C>T variant was ‘possibly pathogenic’ (18). Therefore, the mitochondrial dysfunction, caused by the ND4 m.11778G>A mutation, may be worsened by the m.5601C>T variant. In conclusion, the m.5601C>T variant may have a modified role in clinical expression of LHON-associated m.11778G>A mutation in this family.

Nevertheless, the incomplete penetrance of visual impairment in this family (as evidenced by family members harboring these mutations but exhibiting normal vision) indicated that the ND4 m.11778G>A and tRNA\(^{\text{Ala}}\) m.5601C>T variants are insufficient alone to produce the observed clinical phenotypes. Therefore, it is likely that other risk factors, including environmental factors, nuclear genes and epigenetic

Table V. Pathogenicity scoring system for the m.5601C>T mutation.

| Scoring criteria                                      | m.5601C>T mutation | Score | Classification       |
|-------------------------------------------------------|--------------------|-------|----------------------|
| More than one independent report                       | Yes                | 2     |                      |
| Evolutionary conservation of the base pair             | No changes         | 2     |                      |
| Variant heteroplasmy                                  | No                 | 0     |                      |
| Segregation of the mutation with disease              | Yes                | 2     |                      |
| Histochemical evidence of mitochondrial disease        | Strong evidence    | 2     |                      |
| Biochemical defect in complex I, III or IV            | No                 | 0     |                      |
| Evidence of mutation segregation with biochemical     | No                 | 0     |                      |
| defect from single-fiber studies                      |                    |       |                      |
| Mutant mt-tRNA steady-state level or evidence of       | No                 | 0     |                      |
| pathogenicity in trans-mitochondrial cybrid studies   |                    |       |                      |
| Total score                                           | 8                  |       | Possibly pathogenic  |

Figure 4. Predicted secondary protein structure of tRNA\(^{\text{Ala}}\) with (mutant) and without (wild-type) the m.5601C>T variant (indicated by arrow). The RNA Fold Webserver program (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) was used to predict the structure. The structure is colored according to the base-pairing probability (0-1, as denoted in the color scale bar).
modifications, may contribute to the clinical manifestation of LHON in this pedigree. The main limitation of this study is the lack of functional analysis of the tRNAAla m.5601C>T variant. Further studies, such as the use of cytoplasmic hybrid cells carrying the tRNAAla m.5601C>T variant are required to confirm our conclusions and to identify additional contributing risk factors.

Acknowledgements
Not applicable.

Funding
The present study was supported by The Hangzhou Health and Family Planning Commission (grant no. 2015A04), The Hangzhou Bureau of Science and Technology (grant no. 20150633B16), The Zhejiang Provincial Administration of Traditional Chinese Medicine (grant no. 2018ZB082) and The Ministry of Public Health from Zhejiang Province (grant nos. 2013KYA158 and 2018ZH019).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
YD and YFY designed the study. MYL and BHX performed the molecular analysis. YFY collected the samples and performed the clinical examinations. JHL analyzed the data - performed the phylogenetic analysis. JHL and YD designed the study. MYL and BHX performed the molecular analysis. YFY and YD and YFY designed the study.

Ethics approval and consent to participate
The present study was approved by The ethics committee of Hangzhou First People's Hospital (Hangzhou, China). Written informed consent was obtained from all participants, or their parent/guardian, prior to enrollment in the study.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Yu-Wai-Man P, Turnbull DM and Chinnery PF: Leber hereditary optic neuropathy: A model of mitochondrial neurodegeneration. Biochim Biophys Acta 1658: 172-179, 2004.
2. Carelli V, Rugolo M, Sgarbi G, Ghelli A, Zanna C, Baracca A, Lenaz G, Napoli E, Martinuzzi A and Solaini G: Bioenergetics shapes cellular death pathways in Leber's hereditary optic neuropathy. J Med Genet 39: 162-169, 2002.
3. Puomila A, Hämäläinen P, Kivioja S, Savontaus ML, Koivumäki S, Huoponen K and Nikoskelainen E: Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. J Hum Genet 15: 1079-1089, 2007.
4. Yu-Wai-Man P, Griffiths PG, Brown DT, Howell N, Turnbull DM and Chinnery PF: The epidemiology of Leber hereditary optic neuropathy in the North East of England. Am J Hum Genet 72: 333-339, 2003.
5. Yu-Wai-Man P, Griffiths PG and Chinnery PF: Mitochondrial optic neuropathies-disease mechanisms and therapeutic strategies. Prog Retin Eye Res 30: 81-114, 2011.
6. Jia X, Li S, Xiao X, Guo X and Zhang Q: Molecular epidemiology of mtDNA mutations in 903 Chinese families suspected with Leber hereditary optic neuropathy. J Hum Genet 51: 851-856, 2006.
7. Fraser JA, Biousse V and Newman NJ: The neuro-ophthalmology of mitochondrial disease. Surv Ophthalmol 55: 299-334, 2010.
8. Mackey DA, Oostra RJ, Rosenberg T, Nikoskelainen E, Bronte-Stewart J, Poultion J, Harding AE, Govan G, Bolhuis PA and Norby S: Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. J Hum Genet 51: 481-485, 1996.
9. Catarino CB, Ahting U, Gusic M, Iuso A, Repp B, Peters K, Biskup S, von Livonius B, Proksch H and Klopotck T: Characterization of a Leber's hereditary optic neuropathy (LHON) family harboring two primary mtDNA mutations (m.11778G>A and m.14484T>C) of the mitochondrial DNA. Mitochondrion 36: 15-20, 2017.
10. Yu D, Jia X, Zhang AM, Guo X, Zhang Q and Yao YG: Molecular characterization of six Chinese families with m.3460G>A and Leber hereditary optic neuropathy. Neurogenetics 11: 349-356, 2010.
11. Asanad S, Meert E, Tian JJ, Fantini M, Nassisi M and Sadun AA: Leber's hereditary optic neuropathy: Severe vascular pathology in a severe primary mutation. Intractable Rare Dis Res 8: 52-55, 2019.
12. Liang M, Jiang P, Li F, Zhang J, Ji Y, He Y, Xu M, Zhu J, Meng X, Zhao F, et al: Frequency and spectrum of mitochondrial ND6 mutations in 1218 Han Chinese subjects with leber's hereditary optic neuropathy. Invest Ophthalmol Vis Sci 55: 1321-1331, 2014.
13. Jiang P, Liang M, Zhang J, Gao Y, He Z, Yu H, Zhao F, Ji Y, Liu X, Zhang M, et al: Prevalence of mitochondrial ND4 mutations in 1281 Han Chinese subjects with leber's hereditary optic neuropathy. Invest Ophthalmol Vis Sci 56: 4778-4788, 2015.
14. Rieder MJ, Taylor SL, Toke VO and Nickerson DA: Automating the identification of DNA variations using quality-based fluorescence re-sequencing: Analysis of the human mitochondrial genome. Nucleic Acids Res 26: 967-973, 1998.
15. Andrews RM, Kubacka I, Chinnery PF, Lightowers RN, Turnbull DM and Howell N: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23: 147, 1999.
16. Ruiz-Pesini E and Wallace DC: Evidence for adaptive selection acting on the tRNA and tRNA genes of human mitochondrial DNA. Hum Mutat 27: 1072-1081, 2006.
17. Ding Y, Xia BH, Zhang CJ and Zhuo GC: Mitochondrial tRNA\textsubscript{Ala}(UUR) C3275T, tRNA\textsubscript{Glu} T4363C and tRNA\textsubscript{Aua} A8343G mutations may be associated with PCOS and metabolic syndrome. Gene 642: 299-306, 2018.
18. Yarham JW, Al-Dosary M, Blackley EL, Alston CL, Taylor RW, Elson JL and McFarland R: A comparative analysis approach to determining the pathogenicity of mitochondrial tRNA mutations. Hum Mutat 32: 1319-1325, 2011.
19. Kong QP, Bandelt HJ, Sun C, Yao YG, Salas A, Achilli A, Wang CY, Zhong L, Zhu CL, Wu SF, et al: Updating the East Asian mtDNA phylogeny: A prerequisite for the identification of pathogenic mutations. Hum Mol Genet 15: 2076-2086, 2006.
20. Bibb MJ, Van Etten RA, Wright CT, Walberg MW and Clayton DA: Sequence and gene organization of mouse mitochondrial DNA. Cell 26: 167-180, 1981.
21. Gadaleta G, Pepe G, De Candia G, Quagliariello C, Sbisa E and Saccone C: The complete nucleotide sequence of the rattus norvegicus mitochondrial genome: Cryptic signals revealed by comparative analysis between vertebrates. J Mol Evol 28: 497-516, 1989.
22. Bock J, Ma DP, Wilson RK and Wong JF: The complete nucleotide sequence of the xenopus laevis mitochondrial genome. J Biol Chem 260: 9759-9774, 1985.
23. Qu J, Li R, Zhou X, Tong Y, Lu F, Qian Y, Hu Y, Mo QJ, West CE and Guan MX: The novel A4435G mutation in the mitochondrial tRNA\textsubscript{Met} may modulate the phenotypic expression of the LHON-associated ND4 G11778A mutation. Invest Ophthalmol Vis Sci 47: 475-483, 2006.

24. Li R, Qu J, Zhou X, Tong Y, Hu Y, Qian Y, Lu F, Mo QJ, West CE and Guan MX: The mitochondrial tRNA(Thr) A15951G mutation may influence the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family. Gene 376: 79-86, 2006.

25. Zhang M, Zhou X, Li C, Zhao F, Zhang J, Yuan M, Sun YH, Wang J, Tong Y, Liang M, \textit{et al}: Mitochondrial haplogroup M9a specific variant ND1 T3394C may have a modifying role in the phenotypic expression of the LHON-associated ND4 G11778A mutation. Mol Genet Metab 101: 192-199, 2010.

26. Qu J, Li R, Zhou X, Tong Y, Yang L, Chen J, Zhao F, Lu C, Qian Y, Lu F and Guan MX: Cosegregation of the ND4 g11696A mutation with the LHON-associated ND4 G11778A mutation in a four generation Chinese family. Mitochondrion 7: 140-146, 2007.

27. Zhang J, Zhou X, Zhou J, Li C, Zhao F, Wang Y, Meng Y, Wang J, Yuan M, Cai W, \textit{et al}: Mitochondrial ND6 T14502C variant may modulate the phenotypic expression of LHON-associated G11778A mutation in four Chinese families. Biochem Biophys Res Commun 399: 647-653, 2010.

28. Qu J, Zhou X, Zhang J, Zhao F, Sun YH, Tong Y, Wei QP, Cai W, Yang L, West CE and Guan MX: Extremely low penetrance of Leber's hereditary optic neuropathy in 8 Han Chinese families carrying the ND4 G11778A mutation. Ophthalmology 116: 558-564, 2009.

29. Florentz C, Sohn B, Tryoen-Toth P, Putz J and Sissler M: Human mitochondrial tRNAs in health and disease. Cell Mol Life Sci 60: 1356-1375, 2003.

30. Wallace DC and Lott MT: Leber hereditary optic neuropathy: Exemplar of an mtDNA disease. Handb Exp Pharmacol 240: 339-376, 2017.

31. Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Eilas LJ II and Nikoskelainen EK: Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242: 1427-1430, 1988.

32. Zhang J, Ji Y, Lu Y, Fu R, Xu M, Liu X and Guan MX: Leber's hereditary optic neuropathy (LHON)-associated ND5 12338T>C mutation altered the assembly and function of complex I, apoptosis and mitophagy. Hum Mol Genet 27: 1999-2011, 2018.

33. Ji Y, Zhang AM, Jia X, Zhang YP, Xiao X, Li S, Guo X, Bandelt HJ, Zhang Q and Yao YG: Mitochondrial DNA haplogroups M7b1’2 and M8a affect clinical expression of leber hereditary optic neuropathy in Chinese families with the m.11778G->a mutation. Am J Hum Genet 83: 760-768, 2008.

34. Ueda T, Yotsumoto Y, Ikeda K and Watanabe K: The T-loop region of animal mitochondrial tRNA(Ser)(AGY) is a main recognition site for homologous seryl-tRNA synthetase. Nucleic Acids Res 20: 2217-2222, 1992.

35. Zheng P, Li S, Liu C, Zha Z, Wei X and Yuan Y: Mitochondrial tRNA\textsubscript{Met} C5601T mutation may modulate the clinical expression of tRNA\textsubscript{Met} A4435G mutation in a Han Chinese family with hypertension. Clin Exp Hypertens 40: 595-600, 2018.

36. Tanaka M, Ino H, Ohno K, Ohbayashi T, Ikebe S, Sano T, Ichiki T, Kobayashi M, Wada Y and Ozawa T: Mitochondrial DNA mutations in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Biochem Biophys Res Commun 174: 861-868, 1991.

37. Ding Y, Teng YS, Zhuo GC, Xia BH and Leng JH: The mitochondrial tRNA\textsubscript{His} G12192A mutation may modulate the clinical expression of deafness-associated tRNA\textsubscript{Thr} G15927A mutation in a Chinese pedigree. Curr Mol Med 19: 136-146, 2019.

38. Zhou HH, Dai XN, Lin B, Mi H, Liu XL, Zhao FX, Zhang JI, Zhou XT, Sun YH, Wei QP, \textit{et al}: The analysis of Leber's hereditary optic neuropathy associated with mitochondrial tRNA\textsubscript{Ala} C5601T mutation in seven Han Chinese families. Yi Chuan 34: 1031-1042, 2012 (In Chinese).