Available online at www.sciencedirect.com
ScienceDirect
Mitochondrion 8 (2008) 205–210
Mitochondrion
www.elsevier.com/locate/mito

T14484C and T14502C in the mitochondrial *ND6* gene are associated with Leber's hereditary optic neuropathy in a Chinese family

Shirong Zhang [a,1], Lejin Wang [a,b,1], Yansheng Hao [b,1], Pengyun Wang [a,d], Ping Hao [e], Ke Yin [a], Qing K Wang [a,c,*], Mugen Liu [a,*]

[a] *Key Laboratory of Molecular Biophysics of Ministry of Education and Center for Human Genome Research, College of Life Science and Technology, Huazhong University of Science and Technology, 9500 Euclid Avenue, Wuhan 430074, China*
[b] *Department of Pediatric Ophthalmology & Strabismus, Peking University Eye Center and the 3rd Hospital, Beijing 100083, China*
[c] *Center for Cardiovascular Genetics, Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA*
[d] *GuiYang College of Traditional Chinese Medicine, GuiYang 550002, China*
[e] *Shang Qiu Eye Hospital, Henan 476000, China*

Received 12 July 2007; received in revised form 2 February 2008; accepted 26 February 2008
Available online 6 March 2008

Abstract

Leber's hereditary optic neuropathy (LHON) is a maternally inherited ocular disease which has been associated with three primary mitochondrial DNA mutations: G3640A, G11778A, and T14484C. In this study, we clinically characterized a Chinese family with complete penetrance of LHON. The patients in the family presented with variable clinical features. By direct DNA sequence analysis, we identified both T14484C mutation and a nearby T to C variant at nucleotide 14502 of mitochondria DNA. The T14502C variant altered I58 to V of the protein ND6, which was present in all patients of the family, but not in four unaffected family members and 200 normal controls. The co-existence of both T14484C mutation and T14502C substitution in all patients from the same LHON family suggests that T14502C may play a synergistic role with the primary mutation T14484C. The two variants together may account for the complete penetrance and absence of marked gender bias and visual recovery in the Chinese LHON family although we cannot exclude the possibility of simultaneous involvement of additional mitochondrial variant(s).

Keywords: Leber's hereditary optic neuropathy; Mitochondrial DNA (mtDNA) mutation; *ND6* gene; Polymorphism

1. Introduction

Leber's hereditary optic neuropathy (LHON) is a rare, maternally inherited neurodegenerative disease of young adults, which leads to rapid, painless, bilateral loss of central vision (Brown et al., 1995; Howell, 1997). LHON was the first mitochondrial disease in which the mutations in mtDNA were identified (Went, 1999). The incidence of LHON is approximately 1 in 50,000. The disease accounts for about 3% of the blindness affecting young males (Jenson, 2000; Scheffler, 2001).

The sequence analysis of the mitochondrial genome of families with LHON indicates that the majority of LHON mutations occur in the NADH gene (ubiquinone oxidoreductase, ND) that encodes the complex I subunit of the respiratory chain, making this respiratory complex specific for development of LHON. Three primary mtDNA missense mutations, including G3640A, G11778A, and T14484C that are located in the ND1, ND4, and ND6 subunit genes, respectively, have been identified as being responsible for LHON (Wallace et al., 1988; Brown et al., 1995; Mackey et al., 1996; Mashima et al., 1998).

* Corresponding author. Address: Key Laboratory of Molecular Biophysics of Ministry of Education and Center for Human Genome Research, College of Life Science and Technology, Huazhong University of Science and Technology, 9500 Euclid Avenue, Wuhan 430074, China. Tel.: +86 27 87792649; fax: +86 27 87794549.
E-mail addresses: qkwang@mail.hust.edu.cn, wangq2@ccf.org (Q.K Wang), lium@mail.hust.edu.cn (M. Liu).
[1] These authors contributed equally to this work.

1567-7249/$ - see front matter © 2008 Elsevier B.V. and Mitochondria Research Society. All rights reserved.
doi:10.1016/j.mito.2008.02.003

Approximately 95% of LHON cases were associated with one of the three primary mutations (Man et al., 2004). These primary mutations were not sufficient for development of LHON and the secondary point mutations that are relevant for the phenotypic expression of LHON appear to have a synergistic, deleterious effect together with the primary mutations.

Here, we report a Chinese family that presented with the clinical features of LHON. We screened the proband in the family for the presence of one of the three primary LHON mutations. Direct DNA sequence analysis identified the T14484C mutation as well as another nearby T to C variant at position 14502 of mtDNA. The T14502C variant may interact with the primary mutation to cause severe LHON in the family (Ozawa et al., 1991; Qian et al., 2005).

2. Materials and methods

2.1. Study participants

A four generation, Chinese family with clinically diagnosed LHON was enrolled in this study in Jiangxi province of PR China (Fig. 1). The members of the family (II-3, III-1–III-6, IV-3–IV-5, IV-1) who participated in the study gave informed consent. The family members were evaluated by the medical record, the history of eye symptoms or visual disturbances, and detailed clinical exams including best-corrected visual acuity (BCVA), far and near binocular visual acuity, color vision, visual fields tests, and slit-lamp microscopic exams.

2.2. Mutational analysis

Genomic DNA was extracted from the peripheral blood with DNA Isolation Kit for Mammalian Blood (Wizard Genomic DNA Purification kit, Promega, USA) according to the manufacturer's instruction. The presence of the G3460A, G11778A, and T14484C mutations was examined by direct DNA sequence analysis. Briefly, DNA fragments spanning these mtDNA mutations were amplified by PCR. The corresponding positions on mtDNA for the mutations are 3357–3644 for the G3460A mutation, 11,641–11,961 for the G11778A mutation, and 14,200–14,520 for the T14484C mutation. PCR primers were 5′-GGCATTCCTA ATGCTTACCGA-3′ and 5′-TGATCAGAGGATTGAGT AAA-3′ for G3460A; 5′-AGCCCTCGTACTAACAGCC A-3′ and 5′-GGAGTATACGGCTGTGACTA-3′ for G11 778A; 5′-TCAACCAGTAACTACTACT-3′ and 5′-TT TG GGGGAGGTTATATGG-3′ for T14484C.

The PCR products were purified using the Promega QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA), and sequenced with both forward and reverse primers. DNA sequence analysis was performed using the BigDye Terminator Cycle Sequencing v3.1 kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) (Wang et al., 2005).

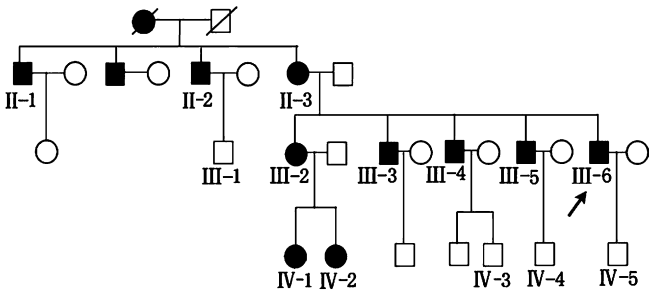

Fig. 1. The pedigree structure of the Chinese family with Leber's hereditary optic neuropathy. The family showed a pattern of maternal inheritance. Individuals with impaired vision are indicated by filled squares (males) or circles (females) and normal individuals are shown with empty symbols. The proband is indicated by an arrow.

3. Result

We characterized a four-generation Chinese family with LHON at Peking University Eye Center, China. Ten family members, including six affected individuals who were diagnosed as having LHON and four unaffected individuals, participated in this study. The proband (III-6) presented with painless, progressive deterioration of bilateral visual impairment at the age of 18. He saw a dark cloud with the central vision. His visual acuity was 0.1 in the right and left eyes. Fundus examinations showed that both of his optic disks were abnormal: vascular tortuosity of the central retinal vessels, a circumpapillary telangiectatic microangiopathy, and no reflex on fovea centralis (Fig. 2). The flash VEP showed bilaterally decreased amplitudes with delayed latencies. Visual field testing revealed a physiologic blind spot which was enlarged with several paracentral scotomas as well as cecocentral scotoma with superior hemisphere visual field defects (Fig. 3A). Visual

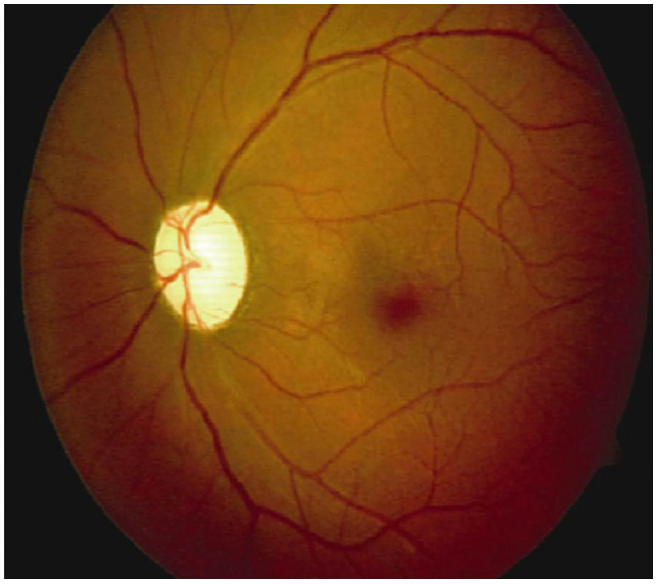

Fig. 2. Fundus photograph of the proband.

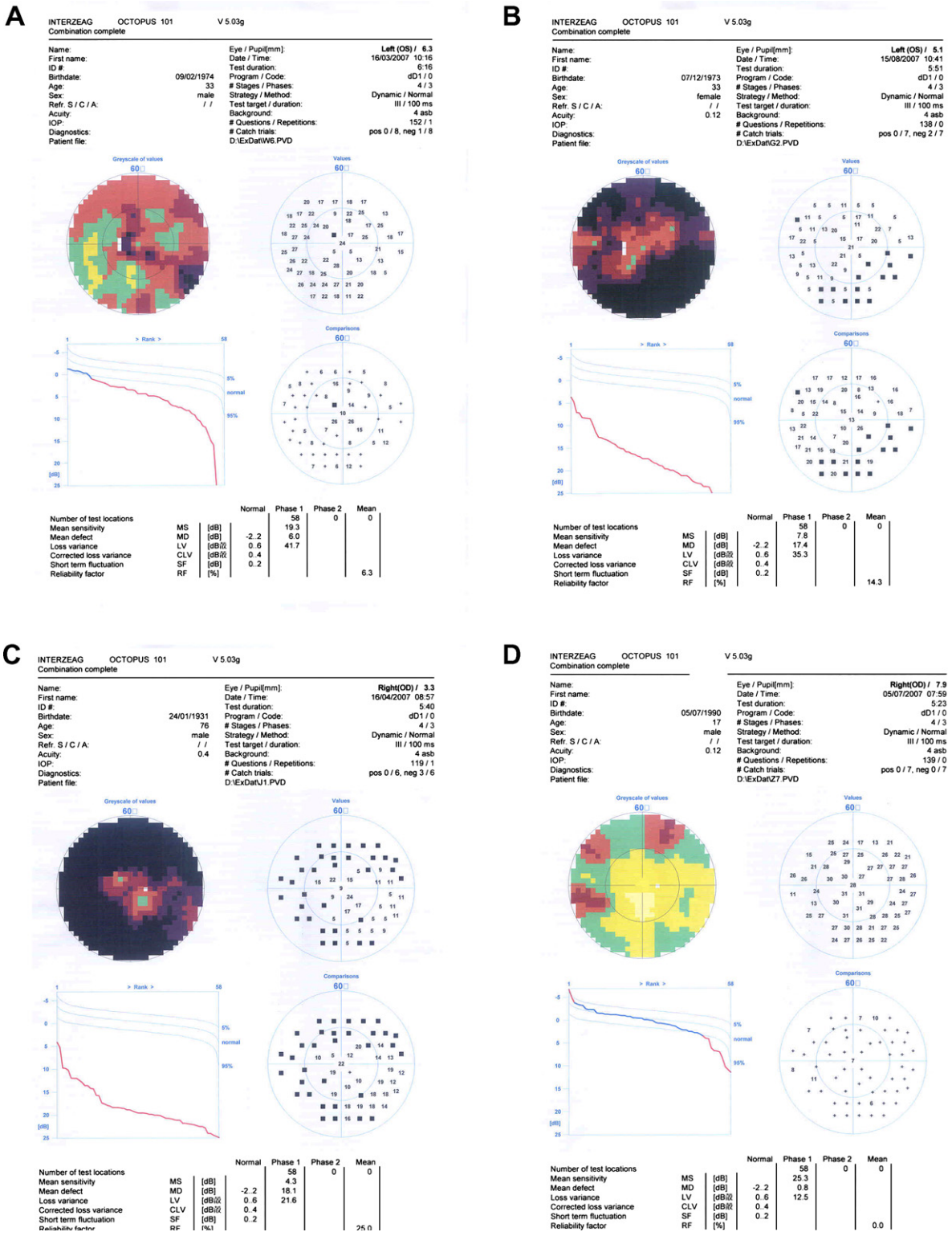

Fig. 3. Clinical characterization of patients with visual field analysis (Octopus 101). (A) proband III-6. (B) III-2. (C) II-3. (D) IV-1. For the identification number of each patient, see Fig. 1.

field analysis also revealed defects in other patients in the family, a tubular visual field in patient III-2 (Fig. 3B) and patient II-3 (Fig. 3C), and cecocentral scotoma with superior hemisphere visual field defects and several nasal peripheral scotomasin in patient IV-1 (Fig. 3D). In the family, all matrilineal relatives exhibited typical clinical

feature of LHON. They showed early onset but not congenital, visual impairment. Visual acuity examinations showed variable severity of visual impairment in the maternal kindred, ranging from severe visual impairment (II-2) to mild visual impairment (IV-2) (Table 1).

To identify the causative mutation in the family, we examined three primary, previously-known LHON-associated mtDNA mutations (G3460A, G11778A, and T14484C). All six affected individuals carried the homoplastic T14484C mutation which results in substitution of the methionine residue by a valine residue. They also carried a T to C variants at position 14502 in the ND6, which is also a homoplastic change causing substitution of 58Ile by Val (Fig. 4). Both changes were not found in the normal members of the family and 200 normal controls. The alignment of amino acid residues in human, bovine, mouse, and *Xenopus laevis* showed that the 58 Ile reside is highly conserved in evolution (Qian et al., 2005).

Table 1
Visual acuity for patients in the Chinese family with LHON

ID of family member	Current age	Age of onset	Current visual acuity	
			OD	OS
II-1	62	18	0.04	0.06
II-2	58	17	0.04	0.04
II-3	56	16	0.08	0.06
III-2	39	17	0.12	0.12
III-3	37	17	0.1	0.12
III-4	35	17	0.1	0.1
III-5	32	16	0.08	0.08
III-6	30	18	0.1	0.1
IV-2	21	18	0.12	0.15

OD, left eye; OS, right eye.

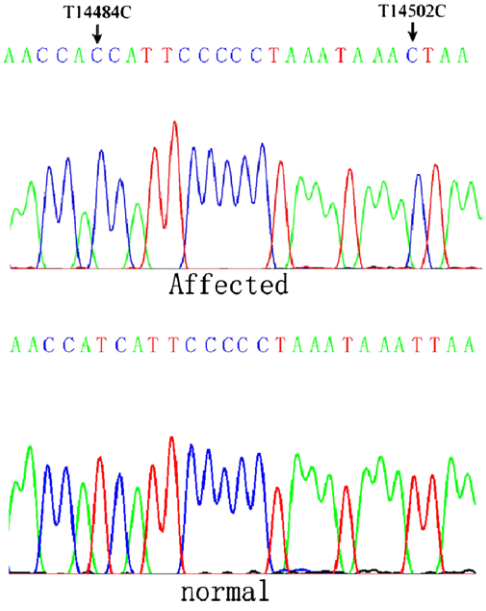

Fig. 4. Identification of the primary T14484C mutation and a nearby T14502C variant in the affected individuals in the Chinese family. Partial sequences of the *ND6* gene (non-coding strand) from the proband and a normal member are shown. The primary mutation T14484C and a secondary T to C nucleotide change at np 14502 are indicated by arrows.

4. Discussion

The mitochondrial *ND6* gene has been considered to be a hot spot for mutations that cause LHON as the 525-bp long *ND6* gene harbors at least nine mutations, including the common mutation at np 14484C (Chinnery et al., 2001; De Vries et al., 1996; Fauser et al., 2002; Gropman et al., 2004; Howell et al., 1998, 2003; Valentino et al., 2002; Wissinger et al., 1997). Protein modeling studies indicate that all of these pathogenic mutations lie within close proximity to one another in a hydrophobic cleft or pocket, and may affect proton translocation or quinine redox catalysis directly (Cardol et al., 2002). It is known from analysis of a frame-shift mutation that the ND6 subunit is essential for the assembly of the membrane arm of complex I and maintenance of its structure (Bai and Attardi, 1998). Recent biochemical studies showed that the T14484C mutation increased the sensitivity of complex I to binding of inhibitors at the ubiquinone site (Carelli et al., 1999). These findings support the speculation that the pathogenic ND6 mutations may cause the defective assembly of the membrane arm and loss of function of entire complex I.

The families carrying T14484C always exhibits marked gender bias, high visual recovery and incomplete penetrance if the age-at-onset is before 20 (Man et al., 2002). The patients in the Chinese family under this study showed variably severe clinical features of LHON. Unlike previous reports that the ratios between affected male and female matrilineal relatives were high, which would be up to 7.7:1 (Man et al., 2002), the ratio in this Chinese family was only 1.4:1, which was close to the ratio of WZ6 family which also carried T14502C substitution coexisting with the primary mutation G11778A in the study by Qian et al. (2005). In our family, the age-at-onset for the proband was 18, and the average age-at-onset was 17 (Table 1). There is evidence that patients with the T14484C mutation are more likely to show improvement if visual loss occurs before the age of 20, while the patients in our study showed no functional recovery. The penetrance of the vision loss was complete in the family. By contrast, in several recent reports, the penetrance of the vision loss in three Chinese pedigrees carrying the T14484C mutation was 27–60% (Sun et al., 2006), 16–60% in six Chinese pedigrees carrying the G11778A mutation, and 35.5% in WZ6 family (Qian et al., 2005; Qu et al., 2005, 2006). The T14484C mutation was also identified in the European and Asian populations, and some mutation carriers did not show any symptoms of Leber's hereditary optic neuropathy (Achilli et al., Palanichamy et al.). The comparison suggests that the T14484C mutation by itself is not sufficient to produce the observed phenotype expressivity consisting of the absence of marked gender bias and visual recovery and the presence of complete penetrance. Functional studies for the mitochondrial defects in lymphoblasts and

transmitochondrial cybrids with the three most common LHON mutations 3460A, 11778A and 14484C revealed that the 14484C mutation was associated with a much milder biochemical defect than the two other mutations (Brown et al., 2000).

Many modifier factors may contribute to different levels of severity of the phenotype, gender bias, the visual recovery rate and variable penetrance rate. These factors include the secondary mutation, mitochondrial haplotypes, nuclear backgrounds, and other environmental factors (Puomila et al., 2007). In our study, the nearby nucleotide change T14502C may play a synergistic effect with primary T14484C mutation. The T to C variant at np 14502 was present in all affected subjects in the family, but not in the normal subjects and 200 normal controls. On the other hand, the T14502C variant is a potential polymorphism as the 58Ile is not conserved and the Val residue at 58 is found in 16 of 61 mammalian species (*Didelphis virginiana*, *Hippopotamus amphibious*, *Nycticebus coucang*, *Tachyglossus aculeatus*, *Vombatus ursinus*, *Dugong dugon*, *Ochotona collaris*, *Pongo pygmaeus*, *Echinops telfairi*, *Ornithorhynchus anatinus*, *Isoodon macrourus*, *Orycteropus afer*, *Thryonomys swinderianus*, *Trichosurus vulpecula*, *Loxodonta Africana*, and *Macaca sylvanus*) (http://mtsnp.tmig.or.jp/mtsnp/search_mtSNP_e.html). Although WZ6 family habouring T14502C substitution also showed low penetrance, that is more likely that the variant was relatively far from the G11778A mutation that decreased its synergy. Thus, we speculate that the T14502C variant may play the synergistically role with the primary T14484C mutation, leading to the complete penetrance of LHON in this family. One limitation of the current study is that the entire mitochondrial genome from the patients was not sequenced. Thus, we cannot exclude the possibility that other variant(s) in other mitochondrial genes may also modify the phenotype conferred by the primary T14484C mutation.

Acknowledgments

We are grateful to our patients for their cooperation in this study. The authors also thank Beijing Third Hospital for excellent clinical support. This study was supported by the 863 Programs of China (2006AA02Z476), National Natural Science Foundation of China (No. 30470975 and 30540030), and grants from the Natural Science Foundation of Hubei Province (No. 2006ABA079).

References

Achilli, A., Rengo, C., Battaglia, V., Pala, M., Olivieri, A., Fornarino, S., Magri, C., Scozzari, R., Babudri, N., Santachiara-Benerecetti, A.S., Bandelt, H.J., Semino, O., Torroni, A., 2005. Saami and Berbers – an unexpected mitochondrial DNA link. Am. J. Hum. Genet. 76 (5), 883–886.

Bai, Y.D., Attardi, G., 1998. The mtDNA-encoded ND6 subunit of mitochondrial NADH dehydrogenase is essential for the assembly of the membrane arm and the respiratory function of the enzyme. EMBO J. 17, 4848–4858.

Brown, M.D., Torroni, A., Reckord, L.C., Wallace, D.C., 1995. Phylogenetic analysis of Leber's hereditary optic neuropathy mitochondrial DNA's indicates multiple independent occurrences of the common mutations. Hum. Mutat. 6, 311–325.

Brown, M.D., Trounce, I.A., Jun, A.S., Allen, J.C., Wallace, D.C., 2000. Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mtDNA mutations. J. Biol. Chem. 275, 39831–39836.

Cardol, P., Matagne, R.F., Remacle, C., 2002. Impact of mutations affecting ND mitochondria-encoded subunits on the activity and assembly of complex I in Chlamydomonas. Implication for the structural organization of the enzyme. J. Mol. Biol. 319, 1211–1221.

Carelli, V., Ghelli, A., Bucchi, L., Montagna, P., De Negri, A., Leuzzi, V., et al., 1999. Biochemical features of mtDNA 14484(ND6/M64V) point mutation associated with Leber's hereditary optic neuropathy. Ann. Neurol. 45, 320–328.

Chinnery, P.F., Brown, D.T., Andrews, R.M., Singh-Kler, R., Riordan-Eva, P., Lindley, J., Applegarth, D.A., Turnbull, D.M., Howell, N., 2001. The mitochondrial ND6 gene is a hot spot for mutations that cause Leber's hereditary optic neuropathy. Brain 124, 209–218.

De Vries, D.D., Went, L.N., Bruyn, G.W., Scholte, H.R., Hofstra, R.M., Bolhuis, P.A., van OoSt, B.A., 1996. Genetic and biochemical impairment of mitochondrial complex I activity in a family with Leber hereditary optic neuropathy and hereditary spastic dystonia. Am. J. Hum. Genet. 58, 703–711.

Fauser, S., Leo-Kottler, B., Besch, D., Luberichs, J., 2002. Confirmation of the 14568 mutation in the mitochondrial ND6 gene as causative in Leber's hereditary optic neuropathy. Ophthalmic Genet. 23, 191–197.

Gropman, A., Chen, T.J., Perng, C.L., Krasnewich, D., Chernoff, E., Tifft, C., Wong, L.J., 2004. Variable clinical manifestation of homoplasmic G14459A mitochondrial DNA mutation. Am. J. Med Genet. A 124, 377–382.

Howell, N., 1997. Leber hereditary optic neuropathy: mitochondrial mutations and degeneration of the optic nerve. Vision Res. 37, 3495–3507.

Howell, N., Bogolin, C., Jamieson, R., Marenda, D.R., Mackey, D.A., 1998. mtDNA mutations that cause optic neuropathy: how do we know? Am. J. Hum. Genet. 62, 196–202.

Howell, N., Oostra, R.J., Bolhuis, P.A., Spruijt, L., Clarke, L.A., Mackey, D.A., Preston, G., Herrnstadt, C., 2003. Sequence analysis of the mitochondrial genomes from Dutch pedigrees with Leber hereditary optic neuropathy. Am. J. Hum. Genet. 72, 1460–1469.

Jenson, B.K., 2000. Nelson Textbook of Pediatrics. W.B. Saunders company, Philadelphia.

Mackey, D.A., Oostra, R.J., Rosenberg, T., Nikoskelainen, E., Bronte-Stewart, J., Poulton, J., Harding, A.E., Govan, G., Bolhuis, P.A., Norby, S., 1996. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. Am. J. Hum. Genet. 59 (2), 481–485.

Man, P.Y., Howell, N., Mackey, D.A., Norby, S., Rosenberg, T., Turnbull, D.M., Chinnery, P.F., 2004. Mitochondrial DNA haplogroup distribution within Leber hereditary optic neuropathy pedigrees. J. Med. Genet. 41, e41.

Mashima, Y., Yamada, K., Wakakura, M., Kigasawa, K., Kudoh, J., Shimizu, N., Oguchi, Y., 1998. Spectrum of pathogenic mitochondrial DNA mutations and clinical features in Japanese families with Leber's hereditary optic neuropathy. Curr. Eye Res. 17 (4), 403–408.

Ozawa, T., Tanaka, M., Sugiyama, S., Ino, H., Ohno, K., Hattori, K., Ohbayashi, T., Ito, T., Deguchi, H., Kawamura, K., Nakane, Y., Hashiba, K., 1991. Patients with idiopathic cardiomyopathy belong to the same mitochondrial DNA gene family of Parkinson's disease and mitochondrial encephalomyopathy. Biochem. Biophys. Res. Commun. 177, 518–525.

Palanichamy, M.G., Sun, C., Agrawal, S., Bandelt, H.J., Kong, Q.P., Khan, F., Yang, C.Y., Chaudhuri, T.K., Palla, V., Zhang, Y.P., 2004. Phylogeny of mitochondrial DNA macrohaplogroup N in India, based on complete sequencing: implications for the peopling of South Asia. Am. J. Hum. Genet. 75 (6), 966–978.

Puomila, A., Hamalainen, P., Kivioja, S., Savontaus, M.L., Koivumaki, S., Huoponen, K., Nikoskelainen, E., 2007. Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. Eur. J. Hum. Genet. 15 (10), 1079–1089.

Qian, Y., Zhou, X., Hu, Y., Tong, Y., Li, R., Lu, F., Yang, H., Mo, J.Q., Qu, J., Guan, M.X., 2005. Clinical evaluation and mitochondrial DNA sequence analysis in three Chinese families with Leber's hereditary optic neuropathy. Biochem. Biophys. Res. Commun. 332, 614–621.

Qu, J., Li, R., Tong, Y., Hu, Y., Zhou, X., Qian, Y., Lu, F., Guan, M.X., 2005. Only male matrilineal relatives with Leber's hereditary optic neuropathy in a large Chinese family carrying the mitochondrial DNA G11778A mutation. Biochem. Biophys. Res. Commun. 328, 1139–1145.

Qu, J., Li, R., Zhou, X., Tong, Y., Lu, F., Qian, Y., Hu, Y., Mo, J.Q., West, C.E., Guan, M.X., 2006. The novel A4435G mutation in the mitochondrial tRNAMet may modulate the phenotypic expression of the LHON associated ND4 G11778A mutation. Invest. Ophthalmol. Vis. Sci. 47, 475–483.

Scheffler, I.E., 2001. A century of mitochondrial research: achievements and perspectives. Mitochondrion 1, 3–31.

Man, P.Y.W., Turnbull, D.M., Chinnery, P.F., 2002. Leber hereditary optic neuropathy. J. Med. Genet. 39, 162–169.

Sun, Y.H., Wei, Q.P., Zhou, X., Qian, Y., Zhou, J., Lu, F., Qu, J., Guan, M.X., 2006. Leber's hereditary optic neuropathy is associated with the mitochondrial ND6 T14484C mutation in three Chinese families. Biochem. Biophys. Res. Commun. 347, 221–225.

Valentino, M.L., Avoni, P., Barboni, P., Pallotti, F., Rengo, C., Torroni, A., Bellan, M., Baruzzi, A., Carelli, V., 2002. Mitochondrial DNA nucleotide changes C14482G and C14482A in the ND6 gene are pathogenic for Leber's hereditary optic neuropathy. Ann. Neurol. 51, 774–778.

Wallace, D.C., Singh, G., Lott, M.T., Hodge, J.A., Schurr, T.G., Lezza, A.M., Elsas 2nd, L.J., Nikoskelainen, E.K., 1988. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242 (4884), 1427–1430.

Wang, Q., Liu, M., Xu, C., Tang, Z., Liao, Y., Du, R., Li, W., Wu, X., Wang, X., Liu, P., Zhang, X., Zhu, J., Ren, X., Ke, T., Wang, Q., Yang, J., 2005. Novel CACNA1S mutation causes autosomal dominant hypokalemic periodic paralysis in a Chinese family. J. Mol. Med. 83, 203–208.

Went, L.N., 1999. Leber hereditary optic neuropathy (LHON): a mitochondrial disease with unresolved complexities. Cytogenet. Cell Genet. 86, 153–156.

Wissinger, B., Besch, D., Baumann, B., Fauser, S., Christ-Adler, M., Jurklies, B., Zrenner, E., Leo-Kottler, B., 1997. Mutation analysis of the ND6 gene in patients with Leber's hereditary optic neuropathy. Biochem. Biophys. Res. Commun. 234, 511–515.