Heteroplasmy of Leber hereditary optic neuropathy and clinical expression in Chinese pedigrees with 11778A mutation

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Objectives To investigate the proportion of 11778A mutation in Chinese individuals with Leber hereditary optic neuropathy (LHON), analyze its relationship with **prevalence (penetrance)** and the severity of visual loss.**Methods** Seven Chinese pedigrees of LHON with 11778A mutation were investigated and clinically evaluated. Peripheral blood was drawn from 96 maternal relatives and the proportion of mutant 11778A was measured by fluorescence-based primer extension and restriction fragment length polymorphism analysis. The relationships between the proportion of mutant 11778A with prevalence and severity of visual acuity were compared. **Results** One hundred maternal relatives (46 male, 54 female) were contained in the 7 pedigrees and 22 male, 15 female were affected. The visual loss can be great difference for members from different pedigrees but may be similar in the same pedigree. In 4 pedigrees, the proportion of mutant 11778A was 100% (homoplasmy) in each of the 55 members. In other 3 pedigrees, which included 41 individuals and 16 of them had symptom and 25 without symptom, except one without symptom had normal genotype, the proportion of mutant 11778A was 27% to 92% (mean 75.1%) in symptomatic members and 12% to 96% (mean 69.1%) in asymptomatic individuals (heteroplasmy). There was no statistical difference between the proportion of mutant mtDNA in these two groups ($P = 0.31$, T test). And there was no relationship between the proportion of mutant mtDNA with the severity of visual loss.**Conclusions** Most individuals who possess similar proportion of 11778A mutation have different prevalence of LHON and severity of visual loss. The proportion of mutant 11778A itself cannot explain the variation in the clinical phenotype of LHON.
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Key Words Leber's hereditary optic neuropathy; 11778A mutation; heteroplasmy; mtDNA

Introduction

Leber hereditary optic neuropathy (LHON) is a maternally inherited blindness occurring predominantly in young adult males. Over 95% patients harbor one of the three point mitochondrial DNA (mtDNA) mutations: 11778A, 14484C, 3460A[1]. All of these mutations affect complex I of the respiratory chain leading to mitochondrial dysfunction and selective damage to the papillomacular bundle of the optic nerve[2]. The disease typically presents with acute or subacute visual loss but the severity of visual loss and the penetrance vary among different pedigrees and even members within the same family. Thus, the presence of one of the primary mutations is a necessary but not sufficient condition for disease development. Other factors such as secondary gene, level of mtDNA mutation and /or environment are thought to take part in the developing of the disease.

Disease causing mutations in mtDNA, unlike neutral polymorphic nucleotides (Lagerstr鯉-Fermér et al., 2001), are typically heteroplasmic with normal and mutant sequences coexisting in the same cell (Wallace, 1999). This is analogous to the heterozygous state in mendelian genetics but because each cell may contain thousands of copies of the mitochondrial genome the level of heteroplasmy can vary from 1% to 99%. Hence, a female harboring a mtDNA mutation may transmit a variable amount of mutated mtDNA to her offspring, which can potentially result in considerable clinical variability amongst siblings within the same family.

Initial reports from Wallace and Vilkki et al found that 11778A mutation was present in all mtDNA molecules of the individuals within the maternal lineage; that is, the individuals were homoplasmic for the 11778 mutation (Wallace et al. 1988; Singh et al. 1989; Vilkki et al. 1989). But subsequent studies recognized (Holt et al. 1989; Bolhuis et al. 1990; Lott et al. 1990; Vilkki et al. 1990; Newman et al. 1991) that, in some pedigrees, the mutant and wild-type mtDNAs coexisted as a heteroplasmic mixture within an individual. Furthermore studies from west countries shown that approximately 15% of LHON pedigrees include family members who are heteroplasmic for the primary LHON mutation [Newman et al., 1991; Smith et al., 1993; Howell et al., 1994]. In recent years, heteroplasmic individuals with the primary mutations of LHON were also found in Asian populations (Chuenkongkaewet al. 2001; Sudoyo et al. 1998, 2002). The aim of present study was to report 3 heteroplasmic

pedigrees of LHON with 11778A mutation in central China and study the correlation between the proportion of mutant mtDNA and its clinical expression.

Material and Methods

Pedigrees selection

During 2004 and 2005, 12 pedigrees of LHON that had the typical clinical neuro-ophthalmic features were examined in our hospital for the scanning of 3460A, 14484C, and 11778A mutations with polymerase chain reaction (PCR), single strand conformation polymorphism (SSCP) and DNA sequencing. All of the family members are Han Chinese of Henan Province (Central China). Results showed that 10 pedigrees had 11778A mutation, one 3460A mutation and no primary mutation in the other family. After discussion with them, the maternal relatives in 7 pedigrees (six 11778A and one 3460A) received further study for proportion of mutation. Heteroplasmy was found in 3 pedigrees of 11778A mutation.

All the study was approved by the Zhengzhou University Ethics Committee. Clinical ocular examination was performed by an ophthalmologist and data of age, gender, age at onset, maternal relation, and best corrected visual acuity (BCVA) were recorded. For statistical analysis, BCVA was converted to logMAR (logMAR = log 1/Snellen visual acuity). The unmeasured visual acuities were at the following logMAR values: counting fingers corresponding to Snellen visual acuity 6/600 (logMAR = 2.0) and hand motion corresponding to Snellen visual acuity 6/6000 (logMAR = 3.0).

Quantification of mutant mtDNA

Whole blood samples were obtained with informed consent. Total genomic mtDNA was extracted using standard techniques. The proportion of mutant mtDNA was quantified by fluorescence-based primer extension and restriction fragment length polymorphism analysis[9]. A polyactylamide gel-purified 5'-JOE-labeled nested primer with the sequence 5'–GCGAGGCTTGCTAGAAGT-3' (nucleotides 11836 -11853 of the H-strand) and Vent (exo-) DNA polymerase in the primer extension reaction was used. The primer extension products, a 214-bp fragment for the sample, are restricted with the endonuclease Tsp45 I (New England Biolabs, Schwalbach, Germany), which cleaves the wild-type mtDNA into 138-bp and 76-bp fragments. The mutant mtDNA remains uncleaved. The level of mutation was measured in at least three independent reactions and averaged for each DNA sample using PCR-amplified DNA as a template.

Results

Pedigrees information

In the 3 heteroplasmic pedigrees, Pedigree A consisted of 32 maternal members, including 11 affected persons (6 males and 5 females) and 21 carriers (7 males and 14 females); Pedigree B consisted of 7 maternal members, including 4 affected persons (1 male and 3 females) and 3 carriers (0 male and 3 females); and Pedigree C also consisted of 7 maternal members, including 4 affected persons (2 males and 2 females) and 3 carriers (1 male and 2 females). Because the earliest age of onset of LHON was 5 years old, a girl in Pedigree A younger than that of age was excluded this study. Also in Pedigree A, three asymptomatic relatives (2 males and 1 female) older than 75 years refused to do examination and 1 symptomatic man has been died. Thus, 41 maternal members were studied here.

Of the 41 maternal members, 18 (43.9%) were symptomatic and 23(56.1%) were asymptomatic. The onset age of LHON was from 5 to 31 years old, but most between 13 to 21 years and the averaged age was 17.5 years. The age at onset of visual loss ranged from 5 to 30 years (mean = 15.9 years) in women and from 9 to 31 years (mean = 19.6 years) in men. Though the average onset age of LHON in male is higher than that of the female, there is no statistical difference between them ($P = 0.33$, T test). In the symptomatic individuals, there were 8 males and 10 females; in the asymptomatic ones, 6 males and 17 females. The corrected incidence ratio between male and female was 2.23:1.

The proportion of mutant mtDNA

In the 41 individuals, the proportion of the mutant mtDNA ranged from 0% to 100%, 46% to 87% and 27% to 92% in Pedigree A, B and C respectively. The proportion of the mutant mtDNA was 27% to 100%(mean = 71.11%) in the 18 symptomatic members, and 0% to 96%(mean= 65.48%) in the 23 asymptomatic individuals. There was no statistical difference between the proportion of mutant mtDNA between this two groups ($P = 0.408$, T test).

In the 14 male and 27 female individuals, the proportion of mutant mtDNA ranged from 27% to 82% (mean = 68.2%) and 0% to 96% (mean = 68.9%).

The proportion of mutant mtDNA was also no statistical difference between these two groups ($P = 0.79$, T test). In pedigree A, B and C, the proportion of mutant mtDNA in affected individuals ranged from 62% to 100% (mean = 76.5%), 59% to 68% (mean = 64%), and 27% to 92% (mean = 64.8%) respectively. There was no statistical difference for the proportion of mutant mtDNA among these 3 pedigrees (P=0.31, F Test). Furthermore, in 18 affected persons, the proportion of mtDNA was 62% to 92%(mean=75.1%) in females and 27% to 100% in males. The load levels between them also had no significant

diffenrence(P=0.188, T test). All of the above results showed that there was no significant correlation between the proportion of mutant mtDNA and the onset of disease.

Relationship between proportion of mutant mtDNA and severity of visual acuity loss

Although every affected people had 11778A mutation, their visual acuity loss varied widely, especially among individuals from different pedigrees. But individuals from the same pedigree always had similar corrected vision (Table 1). In Pedigree A, the BCVA in symptomatic cases was from 0.70 to 3.0 logMAR(mean=1.39 logMAR), but in Pedigree B and C, they were 0 to 1.4 logMAR(mean=0.28 logMAR) and 0.04 to 0.70 logMAR(mean=0.46 logMAR) respectively. Correspondence analysis showed there was no correlation between the proportion of mtDNA with visual acuity (r=0.439, P=0.07).

Table 1. Proportion of mtDNA (%) and visual acuity

	Pedigree A									Pedigree B					Pedigree C			
Pro	62	76	67	74	84	74	77	89	100	62	59	64	65	68	57	92	27	83
BCVA	2	1	1.40	0.70	1.22	0.82	0.92	0.82	3	2	0	0.40	1.40	0.30	0.40	0.70	0.04	0.70

Pro: proportion; BCVA: logMAR = log 1/Snellen visual acuity

Discussion

The incomplete penetrance of LHON and its predilection for males to lose vision imply that additional genetic factors (such as nuclear genes and mtDNA background role) and environmental factors must modulate the clinical expression. Alternatively, the gender bias could also result from a combination of subtle anatomical, hormonal, and/or physiological variations between males and females.25 56 91. In these additional factors, heteroplasy has been extensive studied in east countries, especially in west Europe. Reports from Newman and Smith showed that the majority of LHON patients and family members carry homoplasmic mutant mtDNA, only approximately 14% of LHON patients harbor a mixture of mutant and wild-type mtDNA (Newman et al., 1991; Smith et al., 1993). Jacobi et al studied 167 families of LHON, the prevalence of heteroplasmy for the 11778A, 3460A and 14484C mutations was 5.6%, 40% and 36.4%, respectively (Jacobi et al., 2001). Furthermore, the relationship between mutation load, or threshold, in the blood cells and the risk of developing optic atrophy was studied. In the study of Smith, they estimated that the threshold is 75% to 80%, mutation load higher than this level has a higher risk of visual loss. (Smith et al., 1993). Chinnery et al. (2001b) analyzed 17 pedigrees of LHON with 11778A mutation, in addition to clinical expression, he also found that heteroplasmy influences the inheritance of the 11778 mtDNA mutation. The frequency of blindness in males was related to the mutation load that individual's blood. Mothers with 80% mutant mtDNA in blood were less likely to have clinically affected sons than mothers with 100% mutant mtDNA in their blood. However, in the family presented by **Huoponen,** the proband had only 50% of mutantion in his blood but had severe LHON. Jacobi et al also found an affected male patient with 3460C mutation only harbored 26% in his blood cells. In another study, Black et al. (1996) reported that the amount of mutated mtDNA was only 15% in white blood cells of one affected patient with the 3460 mutation. Because all of the 3 primary mutations will affect the respiratory chain complex I, the level of mtDNA heteroplasmy was examined with respect to biochemical effect of the 3460 mutation by Kaplanova et al(Kaplanova et al., 2004). Results showed there was no direct correlation between the mutation load and the biochemical defect. Thus，so far, we cannot detect the persons with risk for optic atrophy by the proportion of mtDNA mutation .

In this study, heteroplasmy of mtDNA mutation was found in 3(42.9%) of the 7 pedigrees of LHON in peripheral blood leukocytes. The heteroplasmic level is nearly 3 times of that white individuals, but similar to the 37% reported in Thai populations. High prevalence of heteroplasmy was also reported in other studies and different mtDNA background was thought to be the causes (Mroczek-Toñska, 2002, Man, 2003, Phasukkijwatana, 2006). In the 41 analyzed heteroplasmic individuals, 18 individuals had symptom and 23 were carriers, the penetrance is 43.9%. It is difficult to compare this result with those reported previously because most of them included both homoplasmic and heteroplamic families. In the 18 symptomatic patients, the proportion of mutant mtDNA was 27% to 100% (mean =76.67%), and 0% to 96% (mean= 65.47%) in the 23 asymptomatic individuals. Although the mean proportion in symptomatic group is higher than that of asymptomatic one, the difference of mean levels between them is not significantly different. Persons could be affected with load of mutant mtDNA from as low as 27% to as high as 100%. Between the 8 symptomatic male and 10 female, the proportion of mutant mtDNA was 67.4% and 75.1% separately, and there was no singnificant difference between them. All of these results showed that there is no direct relation between proportion of mutant mtDNA and penetrance.

In most reports of LHON with 11778A mtDNA mutation, visual acuity loss is usually severe and

permanent, and the correlation between the proportion of mutant mtDNA and the likelihood of visual loss also existed in some studies. One study found that the median final acuity is 1/60[13], and vision acuity recovery in 11778A LHON patients is rare. In our study, although all the affected patients had 11778A mutation, the severity of visual acuity varied considerably. This phenomenon was more obvious between pedigrees. In Pedigree A, visual acuity was lower than 6/60 in half of the 10 patients, and the best visual acuity was 12/60. But in pedigree B and pedigree C, 7 of 8 patients had visual acuity of equal or better than 12/60, and the best one was 60/60. However, the proportion of mutant mtDNA among them was similar. That is to say, there is no correlation between proportion of mutant mtDNA and the severity of visual acuity loss.

In summary, although the validity of this pilot is limited by its small size, all data suggest that heteroplasmy itself cannot be responsible for the differences in the expression of LHON. More studies with larger sample size are needed to understand the role of heteroplasmy in the clinical manifestation.

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