Mitochondrion 36 (2017) 21–28

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Whole mitochondrial genome analysis in South Indian patients with Leber's hereditary optic neuropathy

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ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form 22 October 2016
Accepted 26 October 2016
Available online 28 October 2016

Keywords:
Leber's hereditary optic neuropathy
Mitochondria
Mutation
Haplogroup
South India
Primary mutation

ABSTRACT

Leber's hereditary optic neuropathy (LHON) is a mitochondrial DNA (mtDNA) associated neurodegenerative disorder of retinal ganglion cells. In this study, whole mitochondrial genome sequencing of 75 LHON patients and 40 controls was performed to identify the mutation frequency and haplogroup background of South Indian population. Analysis of mtDNA revealed 559 different variants in LHON patients, including 7 pathogenic mutations, 30 private, and 22 other disease associated variants. A significantly higher ($p = 0.0008$) overall variation load per individual was noted among LHON patients versus controls. We reported for the first time, the association of M haplogroup ($p = 0.028$) with LHON in this cohort.

1. Introduction

Leber's hereditary optic neuropathy (LHON; OMIM 535000) is a rare, mitochondrial DNA (mtDNA) associated neurodegenerative disorder that results in acute or subacute visual loss due to selective degeneration of retinal ganglion cells (Newman and Wallace, 1990; Newman et al., 1991; Carelli et al., 2004). In particular, the P-ganglion cells show preferential degeneration in LHON as they are highly sensitive to metabolic insults and mitochondrial dysfunctions (Sadun et al., 2000; Yu-Wai-Man et al., 2009). The onset of LHON is usually between 15 and 35 years of age and the primary clinical manifestation of this maternally inherited disorder is painless and progressive loss of central vision in both eyes (Newman and Wallace, 1990; Newman et al., 1991). In majority of LHON cases, visual dysfunction is bilateral, the fellow eye becoming affected either simultaneously (25%) or sequentially (75%), with a median inter-eye delay of 6–8 weeks (Harding et al., 1995). Visual acuity reaches a nadir 4–6 weeks after disease onset and it is severely reduced to 6/60 or less (Riordan-Eva and Harding, 1995). The defining feature of this disorder is visual failure, but other symptoms like cardiac arrhythmias and neurological abnormalities such as postural tremor, peripheral neuropathy, nonspecific myopathy and movement disorders are also not uncommon (Nikoskelainen et al., 1994; Nikoskelainen et al., 1995; Meire et al., 1995; Mashima et al., 1996; Bower et al., 1992).

LHON is mainly due to three point mutations in mtDNA: m.3460G>A, m.11778G>A and m.14484T>C; which occurs in the genes encoding complex 1 (NADH dehydrogenase) subunits of the respiratory chain. Over 95% of LHON pedigrees harbor at least one of the three primary mtDNA mutations in vast majority of the populations (Yu-Wai-Man et al., 2009). Epidemiological studies have estimated the prevalence range of LHON from 1/39,000 to 1/50,000 in different populations (Spruijt et al., 2006; Puomila et al., 2007). While, these three common mutations are prerequisites for LHON, only ~50% of male and ~10% of female matrilineal relatives carrying one of the three mutations develop optic neuropathy (Kirkman et al., 2009). This incomplete penetrance and male preponderance in LHON reflects the complex etiology of the disease and indicates that some additional factors, such as mtDNA background, other mtDNA mutations, nuclear genes and environmental factors must be associated with this disorder. To date, 14 other primary pathogenic mtDNA mutations have been reported (http://www.mitomap.org/MITOMAP/MutationsLHON), representing <5% of LHON cases. Previous studies have also suggested that specific mtDNA-haplogroup background and several so-called "secondary mtDNA mutations" in LHON patients can influence the disease penetrance and pathogenic potential of primary mutations (Torroni et al., 1997; Carelli et al., 2006; Hudson et al., 2007). An important meta-analysis study using 159 European LHON pedigrees revealed that the risk of visual loss was greater when the m.11778G>A and m.14484T>C mutations arose on haplogroup J, whereas individuals with the

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http://dx.doi.org/10.1016/j.mito.2016.10.006
1567-7249/

m.3460G>A mutation were more likely to experience visual loss if they belonged to haplogroup K. In contrast, individuals with the m.11778G>A mutation had a lower risk of visual loss when the mutation arose on haplogroup H (Hudson et al., 2007). Moreover, unlike European countries, mutational screening in some populations has shown that the common primary mutations do not account for the vast majority of LHON cases (Jia et al., 2006; Kumar et al., 2010; Kumar et al., 2012; Sundaresan et al., 2010; Khan et al., 2013). The three common mutations are only responsible for 38.3% cases in a large cohort of Han Chinese subjects with LHON (Jia et al., 2006). A study on North Indian subjects demonstrated only 20% of LHON cases had the three primary mutations (Kumar et al., 2010). A report from our laboratory showed the prevalence of these mutations in only 12% of LHON families from Southern India (Sundaresan et al., 2010). These studies collectively suggest the occurrence of a different set of LHON-causing mutations in the mitochondrial genomes of these populations.

In an effort to generate a more comprehensive mutational landscape, in the present study, we performed whole mitochondrial genome sequencing in 75 LHON and 40 age-matched control subjects and explored the haplogroup background and relative frequency of mitochondrial mutations in South Indian LHON cases.

2. Materials and methods

2.1. Study participants

The study protocol was approved by the Institutional Review Board of Aravind Eye Hospital and was conducted in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all study participants after explaining the nature of the study. 75 unrelated clinically diagnosed LHON cases from South India, their available family members, and 150 age- and ethnically-matched healthy controls were recruited from the Neuro-ophthalmology Clinic of the Aravind Eye Hospital, Madurai, India. Participants were interviewed to identify both personal and family medical histories of vision impairments and other clinical anomalies. All participants underwent a comprehensive ophthalmic examination, including visual acuity measurement, visual field evaluation, dilated fundoscopy, and visual evoked potentials. Diagnosis of LHON was based on vision loss, fundus examination showing circumpapillary telangiectatic microangiopathy and swelling of nerve fiber layer around the disc. Imaging of the central nervous system was used in all probands to rule out compressive, infiltrative, and inflammatory causes of optic neuropathy (either computed axial tomography or magnetic resonance imaging). All probands also had routine blood cell assessment to exclude an inflammatory disease process (total count, differential count and erythrocyte sedimentation rate).

2.2. Polymerase chain reaction (PCR) and whole mtDNA sequencing

Peripheral blood samples (5 mL) were collected from each participant, and genomic DNA was extracted using the modified salt precipitation method (Miller et al., 1988). The entire mitochondrial genome was amplified using 24 primer pairs as described previously (Rieder et al., 1998). PCR was performed in ASTEC Gradient thermocycler (Fukuoka, Japan) in a total volume of 20 μL, containing 1× PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; and 0.001% gelatin), 200 μM of dNTPs (Medox Biotech India Pvt. Ltd., Chennai, India), 0.5 pmol of each primer, 50–100 ng of genomic DNA and 1 unit of Taq DNA polymerase (Sigma, Saint Louis, MO). Thermal cycling conditions were 5 min at 95 °C, followed by 35 cycles [1 min at 95 °C, 1 min at the annealing temperature of the primers (56 °C–61 °C) and 1 min at 72 °C] and a final extension for 5 min at 72 °C. PCR products were eluted from agarose gel and column purified using EZ-10 spin column DNA gel extraction kit (Bio Basic Inc., East Markham Ontario, Canada). Bidirectional sequencing was performed using Big Dye Terminator ready reaction mix and analyzed on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.3. Data analysis

The mitochondrial DNA sequences from LHON and control samples were compared with the Revised Cambridge Reference Sequence (rCRS; NC_012920) (Andrews et al., 1999). The variations in the LHON samples were also compared with Mitomap (http://www.mitomap.org); mtDB (http://www.genpat.uu.se/mtDB) and HmtDB (http://www.hmtdb.uniba.it:8080/hmdb) for their significance. The variations, which were not present in database, were categorized as novel mitochondrial variations. The mtDNA haplogroup was assigned to each individual based on the mutation, using available literatures (www.phylotree.org; mtDNA tree Build 17 (18 Feb 2016) and was validated with MitoTool (http://www.mitotool.org). Analysis of private variants was done using the algorithm described previously (Hagen et al., 2015; Bi et al., 2012). The possible effect of the novel non-synonymous mutations was predicted by Sorting Intolerant From Tolerant (SIFT) (Ng and Henikoff, 2003) and Polymorphism Phenotyping (PolyPhen-2) (Hicks et al., 2011) tools.

2.4. Statistical analysis

All the statistical analysis was done using the GraphPad software (GraphPad QuickCalcs; http://graphpad.com/quickcalcs/). Student's unpaired two-tailed *t*-test was used to compare the means of the two groups. Frequencies between cases and controls were compared to evaluate the associations of haplogroups with LHON using Fisher's exact test. A $p < 0.05$ was considered statistically significant in hypothesis testing and 95% confidence interval (CI) was used to describe the estimation of unknown parameters.

3. Results

3.1. Study samples

75 unrelated LHON probands were recruited in this study comprising of 63 males and 12 females. Among the 75 probands, 9 cases were from pedigrees with a family history of LHON and 66 probands were sporadic. A total of 90 members from 75 families were clinically diagnosed with the LHON phenotype, of whom 21 had profound visual impairment, 35 had severe visual impairment, and 34 patients had mild visual impairment. The mean onset age of visual loss was 22.2 years (SD, 9.5 years; range, 8–46 years). Of the 90 members, fundus anomalies were detected in 98% of the eyes, while 2% eyes had normal fundus. Clinical details of the probands identified with common and rare LHON mutations are listed in Table 1. The mean age of controls of same ethnicity was 24.1 years (SD, 4.26 years; range, 17–35 years). There was no significant difference detected between the mean ages of LHON patient and control groups ($p = 0.22$).

3.2. Whole mitochondrial genome analysis

Whole mitochondrial genome sequencing of 75 LHON cases and 40 controls identified a total of 559 and 350 different variants respectively in patients and controls. The distribution of variants across the whole mitochondrial genome as well as the coding region was similar for both the groups (Tables 2 & 3). Of the 374 coding variations in 75 LHON patients, 30.2% (113/374) were non-synonymous and 69.8% (261/374) were synonymous. Control group harbored 25.2% (61/242) of non-synonymous and 74.8% (181/242) of synonymous variants (Table 3). Although each individual of both patient and control groups exhibited different number of genetic variations, the average number of variations were significantly higher ($p = 0.0008$; 95% confidence