Original Article

Frequency of primary mutations of Leber's hereditary optic neuropathy patients in North Indian population

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Purpose: Leber's hereditary optic neuropathy (LHON) is an inherited optic neuropathy characterized by subacute painless vision loss. The majority of LHON is caused due to one of the three primary mutations in the mitochondrial DNA (m.G3460A, m.G11778A, and m.T14484C). The frequency of these mutations differs in different populations. The purpose of this study is to observe the frequency of three common primary mutations in the North Indian population. **Methods:** Forty LHON patients within the age group of 10–50 years underwent molecular testing for primary mutations. For two patients, testing for mother and other siblings was also carried out, using bidirectional sequencing. **Results:** A total of 11 out of 40 (27.5%) patients were found to be carrying m.G11778A mutation. Siblings of two probands were also positive for the same mutation. In one family, two primary mutations (m.G11778A and m.T14484C) were found in the proband and in the mother as well. **Conclusion:** In this study, 27.5% mutation was detected in North Indian LHON families. These results suggest that m.G11778A mutation is more frequent in this population. The results of the present study are compatible with studies of an Asian population and Northern European population.

Key words: Homoplasmy, Leber's hereditary optic neuropathy, mitochondrial DNA, optic atrophy, primary mutation

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Leber's hereditary optic neuropathy (LHON; OMIM 535000) is a subgroup of optic neuropathy, characterized by acute or subacute vision loss. LHON may be unilateral and might involve the fellow eye sequentially with a median inter-eye delay of around 8 weeks.[1]

LHON can occur at any age but usually manifests in the second or third decade of life with mean age of onset being 20–24 years.[2] It is seen that in almost all LHON patients (>95% of European population), one of three common mutations (m.G3460A, m.G11778A, and m.T14484C) present in the mitochondrial complex 1: NADH dehydrogenase (ubiquinone) subunits 1, 4, and 6 (ND1, ND4 and ND6), respectively, is responsible for the disease, but the frequency of these individual mutations varies within populations. Among the three primary mutations, m.G11778A is the predominant mutation seen in most East Asian[3] and European population.[4] Another recent study from the southern part of India showed a reduced frequency of LHON primary mutations.[5]

The objective of the present study is to observe the frequency of the three common primary mutations for LHON in the North Indian population.

Methods

Patient diagnosis

This was a prospective observational study of forty LHON patients who presented to the Neuro-Ophthalmology Clinic of our center from January 2014 to January 2016. A detailed ophthalmological examination was done in all patients, including visual acuity testing using Early Treatment of Diabetic Retinopathy Study chart, color vision using Ishihara's pseudoisochromatic plates, contrast sensitivity with Pelli-Robson chart, visual field testing using Goldmann/automated perimetry, evaluation of pupillary reactions, fundus examination and visually evoked response (Nicolet Ganzfeld stimulator, Nicolet Inc., Madison, WI, USA), retinal nerve fiber layer thickness (RNFL), and retinal ganglion cell layer thickness measured by optical coherence tomography (4.0.2 Carl Zeiss). Patients were clinically diagnosed to have LHON if there was subacute visual loss, with sequential or simultaneous involvement of both eyes, with fundus features such as vascular tortuosity of central retinal vessels, circumpapillary telangiectasia, swelling of RNFL, or optic disc pallor. Those with other causes of optic neuropathy such as glaucoma, trauma, and exposure to drugs such as ethambutol were excluded from the study. Neuroimaging (magnetic resonance imaging brain and orbits) was done in all patients to rule out compressive, infiltrative, or inflammatory causes of optic neuropathy. All patients were subjected to various tests such as complete hemogram, erythrocyte sedimentation rate, C-reactive protein, chest X-ray, Mantoux test, venereal disease research laboratory, and neuromyelitis optica antibody to rule

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out any infectious/inflammatory process. Patients presenting with duration of <2 weeks were excluded to avoid inadvertent inclusion of patients with retrobulbar neuritis. Those patients with clinical features suggestive of LHON were subjected to mutational analysis. Other clinical variables included age, gender, duration, laterality, inter-eye involvement interval, and history of exposure to oxidants in the form of cigarette smoking or alcohol. Written informed consent was taken from all the patients included in the study. The procedures followed were in accordance with our Institutional Ethics Committee and the Helsinki Declaration of 1975, as revised in 2000. Ethical approval was taken from the Institutional Ethics committee for the study.

Sample collection

The targeted mitochondrial genome was amplified using genomic DNA, in nine overlapping fragments,[6] and Sanger sequencing was carried out using ABI 3130 sequencer (Applied Biosystems). The three primary mutations are present in the ND1, ND4, and ND6 regions of mitochondrial complex 1. Sequencing was carried out specifically for these regions. DNA sequences of patients were analyzed using SeqScape v2.5 software (Applied Biosystems). The changes were then checked on the MITOMAP database using revised Cambridge Reference Sequence with GenBank number (NC_012920).

Results

Clinical characteristics

The present study included forty clinically suspected LHON patients. The baseline clinical characteristics of the patients are shown in Table 1 and the phenotypic features in Table 2. The average age of onset of our patient group was 23 years. The male:female ratio was 39:1. A total of 26 patients (65%) showed simultaneous involvement of both eyes whereas 13 patients (32.5%) had sequential involvement of both eyes. One patient (2.5%) had uniocular visual loss at presentation. A total of 29 patients (72.5%) had a history of smoking, and 28 (70%) had a history of alcohol intake.

Visual function

The mean overall visual acuity was 1.42 ± 0.47. A total of 24 patients (60%) showed sparing of pupillary reactions. Color vision was affected in 37 patients (92.5%) and contrast sensitivity was decreased in 38 patients (95%). All patients showed evidence of central or centrocecal scotoma on visual field testing. Five of 40 patients (12.5%) showed fundus findings suggestive of acute phase of the disease such as hyperemia of the optic disc, vascular tortuosity, and blurring of disc margins, while 35 patients (87.5%) had optic atrophy suggesting chronic phase of the disease.

Mutational analysis

All forty patients were screened for the three most common mitochondrial DNA (mtDNA) mutations (m.G11778A, m.T14484C, and m.G3460A) of which 11 (27.5%) were positive for m.G11778A mutation which was in homoplasmic condition as shown in Fig. 1. Only one patient was found carrying two primary mutations (m.G11778A and m.T14484C) as shown in Table 3. His mother was also found to have heteroplasmy for m.G11778A and homoplasmy for m.T14484C change.

Discussion

LHON is one of the most commonly inherited mitochondrial disorders leading to sequential or simultaneous, subacute vision loss. The mean age of patients in the present study was 22 years, and the results were consistent with previous studies from the various parts of the world.[7-9] However, the age of onset was slightly earlier compared to that reported from the Netherlands[10] and Atlanta,[11] where it was 24.4 and 27.6 years, respectively. The reported age of onset in this study is around

Table 1: Baseline clinical characteristics

Clinical variable	Mean±SD
Age (years)	23.8±6.4
Duration of disease (years)	8.1±9.6
Inter-eye interval (weeks)	8.2±2.1
Time to stabilization of visual acuity (months)	3.2±1.4
Contrast sensitivity	0.59±0.51
VER amplitude (μV)	5.81±1.66
VER latency (ms)	114.78±12.17
RNFL thickness (μ)	112.5±22.31
GCL thickness (μ)	61.65±13.05

VER: Visually evoked response, RNFL: Retinal nerve fiber layer, GCL: Ganglion cell layer, SD: Standard deviation

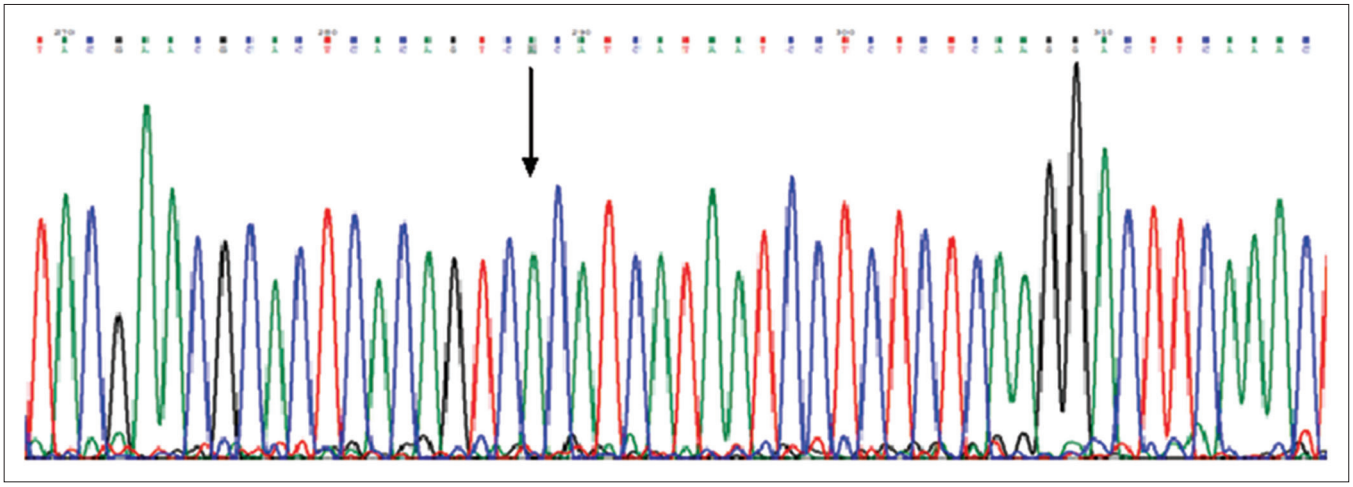

Figure 1: Electrophoretogram showing m.G11778A mutation

Table 2: Phenotypic features

Age (years)	Age of onset (years)	Gender	Visual acuity		Fundus findings disc pallor (yes/no)	Color vision affected (yes/no)	Visual fields
			Right	Left			
11	11	Male	0.47	0.47	Yes	Yes	Central scotoma
16	16	Male	1.77	1.77	Yes	Yes	Centrocecal scotoma
17	16	Male	1.17	1.77	Yes	Yes	Central scotoma
17	17	Male	1	1	Yes	Yes	Centrocecal scotoma
19	19	Female	0.60	0.47	Yes	Yes	Centrocecal scotoma
15	15	Male	1.77	1.30	Yes	Yes	NP
30	30	Male	1.77	1.47	Yes	Yes	NP
14	14	Male	1.77	1.47	No	Yes	NP
33	33	Male	0.47	0.47	Yes	Yes	Centrocecal scotoma
25	19	Male	0.77	0.77	Yes	Yes	Centrocecal scotoma
16	12	Male	1.77	1.77	No	No	NP
22	22	Male	0.77	0.60	Yes	Yes	Centrocecal scotoma
16	14	Male	0.77	0.77	Yes	No	Centrocecal scotoma
38	31	Male	1	2	Yes	Yes	Central scotoma
15	15	Male	1.17	1.77	Yes	Yes	NP
41	40	Male	1.77	1.77	Yes	Yes	NP
28	28	Male	0.47	0.77	No	No	Centrocecal scotoma
17	17	Male	1.7	2.3	Yes	Yes	NP
27	26	Male	2	2	Yes	Yes	NP
27	26	Male	1.77	1.7	Yes	Yes	NP
15	14	Male	1	1	Yes	Yes	Centrocecal scotoma
42	38	Male	1.77	1.77	Yes	Yes	NP
28	27	Male	1.7	1.7	Yes	Yes	NP
38	38	Male	1.07	1.07	Yes	Yes	Centrocecal scotoma
14	14	Male	1	1	Yes	Yes	Centrocecal scotoma
38	37	Male	1.77	1.47	Yes	Yes	NP
30	30	Male	1.17	1.77	Yes	Yes	NP
16	16	Male	1	1	Yes	Yes	Central scotoma
20	19	Male	1.3	1.47	Yes	Yes	NP
12	12	Male	1	0.77	No	No	Centrocecal scotoma
11	6	Male	1	1	Yes	Yes	Centrocecal scotoma
17	17	Male	0.6	1.17	Yes	Yes	Centrocecal scotoma
38	37	Male	0.47	0.47	No	Yes	Centrocecal scotoma
17	17	Male	0.77	0.77	Yes	Yes	Central scotoma
31	29	Male	2	2	Yes	Yes	NP
18	18	Male	0.77	0.77	Yes	Yes	Centrocecal scotoma
23	23	Male	1	1	Yes	Yes	Centrocecal scotoma
20	20	Male	1.77	1	Yes	Yes	Central scotoma
24	21	Male	1.07	0	Yes	Yes	Central scotoma
35	35	Male	0.47	0.47	Yes	Yes	Centrocecal scotoma

NP: Test was not possible due to poor vision

20–24 years,[11] but the loss may appear early in childhood or late adulthood. The present study also observed predominance of males consistent with previous studies of various ethnicities. However, the percentage of males affected (95%) was considerably higher than that reported from European, North American, and Australian families (77%–90%).[12-14] An additional factor for the greater number of males in our study could be financial and geographical distances that limit women in India from accessing tertiary care. Fully 65% of our patients showed simultaneous involvement of both eyes. This is comparable to numbers reported by other studies (64%–70%).[10] The time to stabilization of visual acuity was 3.2 months, which is consistent with other studies as well.[11] More than 95% cases of LHON are caused due to one of the three common primary mutations (m.G3460A, m.G11778A, and m.T14484C). The literature review shows that the frequency of these mutations varies considerably in different population[15] as shown in Table 4. In our study, m.G11778A was the most prevalent.

Table 3: Genotypic characteristics of the patients

Number	Age	Gender	m.3460 G>A	m.11778 G>A	m.14484 T>C
1	11	Male	-	+	+
2	16	Male	-	+	-
3	14	Male	-	+	-
4	25	Male	-	+	-
5	16	Male	-	+	-
6	15	Male	-	+	-
7	17	Male	-	+	-
8	27	Male	-	+	-
9	15	Male	-	+	-
10	42	Male	-	+	-
11	18	Male	-	+	-

-: Mutation negative, +: Mutation positive

Table 4: Frequency of primary mutations in different population

Country (population)	ND1/G3460A (%)	ND4/G11778A (%)	ND6/T14484C (%)	Reference
Finland	11	53	3	[21]
Northern Europe, the United Kingdom, and Australia	13	69	14	[22]
Danish population	13	67	18	[23]
Han Chinese	Nil	35.36	Nil	[24]
Japanese	4	87	9	[25]
Korean	1	56	16	[26]
India (South)	Nil	8.9	3.3	[5]
Present study	Nil	27.5	Nil	

From India, a recent study on the South Indian population showed 12% frequency (11 out of 90 patients) for LHON primary mutations of which 8.9% was detected in m.G11778A, 3.3% for m.T14484C, and none for m.G3460A. In our study, we have found mutations in 27.5% (11 out of 40) of cases and m.G11778A was the only one found. Only, one patient was found to carry two primary mutations (m.G11778A and m.T14484C), and the other positive patients carried the same m.G11778A mutation. There are a few case studies which also show patients carrying two primary mutations (m.G11778A and m.T14484C).[16] In these families, m.T14484C mutation was in homoplasmic condition and m.G11778A in heteroplasmic condition, whereas in our patient, both mutations were in homoplasmic condition. Our results suggest that for the North Indian population studied, m.G11778A seems to be a more prevalent cause for LHON than other primary mutations.

"Secondary" mutations are also sometimes seen in LHON patients, but their pathogenic effect is as yet unclear. It is seen that isolated secondary mutation may not cause LHON, but with the presence of any one of the primary mutations, it may play a role in the development of the disease.[15] There are studies where secondary mutations were found to be associated with disease manifestations, but these are mostly seen to occur in single cases.[17] However, in our study, due to targeted screening for primary mutations, the presence of secondary mutations cannot be ruled out. In addition, 26 patients were seen to carry m.G11719A change, which was reported as polymorphism[18] and may not be involved in disease manifestation.

If targeted testing for common mutations does not identify a pathogenic variant and the clinical suspicion remains high, then complete mtDNA sequencing should be considered. A few other mtDNA changes have been reported in a small number of cases (m.G3700A, m.G3733A, m.G3733C, m.C4171A, m.T10663C, m.G14459A, m.C14482A, m.A14495G, and m.C14568T)[19] in which LHON was the diagnosis, made based on high clinical index. Although the pathogenic significance of these variants has not been proven, in some populations, they have been observed to make up a significant fraction of LHON cases and thus are presumed to be responsible for LHON.[19] Some authors have suggested that when these secondary mutations are present along with primary mutations, they may be causative of LHON.[20]

Being a heterogeneous disorder, mitochondrial disease poses a diagnostic challenge. There can be certain presentations that mimic similar conditions. Since the objective was to study the frequency of three common primary mutations, we did not screen for any other changes.

Conclusion

We reported a frequency of 27.5% of common primary mutation for LHON in the studied North Indian population. Since we have found only one mutation (m.G11778A), we presume that this may be the most prevalent in the northern part of the country. Similar studies involving whole mitochondrial sequencing are required to determine whether there are any secondary mutations involved that have a synergistic effect on the primary mutations or whether

secondary mutations by themselves might cause a similar phenotype.

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Conflicts of interest

There are no conflicts of interest.

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