Genetics

# Leber's Hereditary Optic Neuropathy–Specific Mutation m.11778G>A Exists on Diverse Mitochondrial Haplogroups in India

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**Purpose.** Leber's hereditary optic neuropathy (LHON; OMIM 535000) is one of the most common maternally inherited mitochondrial disorders. Three mitochondrial DNA point mutations—m.3460G>A (MT-ND1), m.11778G>A (MT-ND4), and m.14484T>C (MT-ND6)—account for the majority of reported LHON cases. Only approximately 50% of males and approximately 10% of females carrying these mutations develop optic neuropathy and blindness. Additional factors, such as mtDNA/nuclear genetic background and environmental modifiers, are likely to contribute toward the observed incomplete penetrance and gender bias. We aimed to investigate whether mtDNA haplogroup influences LHON clinical expression in Indian patients harboring the m.11778G>A mutation.

**Methods.** Detailed clinical assessment and complete mitochondrial genome sequencing was undertaken in 64 LHON families harboring the m.11778G>A mutation. Mitochondrial haplogroup was assigned based on evolutionarily conserved mtDNA variations.

**Results.** A total of 543 individuals (295 male, 248 female) from 64 unrelated families harboring the m.11778G>A mutation were recruited to the study. The overall disease penetrance was 27.07% (146 of 543) and higher in males (37.9%; 112 of 295) than females (13.7%; 34 of 248). The mtDNA haplogroup analysis revealed that all affected probands belonged to different mtDNA haplogroups. No association between the m.11778G>A mutation and the background mtDNA haplogroup was detected.

**Conclusions.** The first detailed study of Indian LHON patients confirm that the m.11778G>A-related LHON in India coexists with multiple different mtDNA haplogroups, unlike the preferential association of west Eurasian haplogroup J and the reported increased clinical penetrance with the J2 subhaplogroup. However, we observed variable penetrance of LHON in different Indian mtDNA haplogroup backgrounds, indicating their possible influence on clinical expression. These data suggest that a similar heterogeneity, resulting from the mtDNA haplogroup, might also exist in other mitochondrial diseases among Indian populations.

Keywords: LHON, mtDNA, mutations, m.11778G>A, haplogroup

Leber hereditary optic neuropathy (LHON; OMIM 535000) is the most common maternally inherited mitochondrial disorders.[1,2] Clinical features include the slowly progressive loss of central vision with apoptotic death of retinal ganglion cells and optic nerve degeneration.[3,4] Age of onset varies from adolescence to young adults, with late-onset disease also reported.[5] More than 95% of the reported LHON results from one of three primary mitochondrial DNA (mtDNA) point mutations (m.3640G>A, m.11778G>A, and m.14484T>C) in nicotinamide adenine dinucleotide - hydrogen dehydrogenase (ND) subunits of the mitochondrial complex I.[6,7] Although additional mtDNA mutations are reported to cause LHON, these are relatively rare within the population.[1] Interestingly, not all individuals who inherit these primary mtDNA mutations

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develop optic neuropathy and loss of vision, consistent with variable penetrance among different pedigrees.[8,9] Furthermore, only approximately 50% of the males and approximately 10% of the females carrying these mutations manifest visual symptoms.[10] These observations suggest additional factors contribute toward disease expression,[11–13] including mtDNA haplogroup and heteroplasmy, nuclear background, and environment factors, such as smoking and alcohol consumption.[8,9,14–18]

The role of the mtDNA genetic background and its association on primary mutations was reported in 1997.[13,19,20] Subsequent studies suggested that the m.11778G>A and m.14484T>C mutations preferentially associate with the Western Eurasian mtDNA haplogroup J.[13,19–21] A detailed meta-analysis confirmed that individuals harboring the m.14484T>C and m.11778G>A mutations were more likely to belong to the Western Eurasian haplogroup J than control subjects (27- and 3-fold, respectively).[6] These observations also implicated the involvement of other J haplogroup defining motifs, such as m.4216T>C and m.13708G>A, with an increased risk of disease expression.[13]

Evidence of the influence of the mtDNA haplogroup on the clinical expression of LHON in European families was elucidated by Hudson et al.[8] The risk of visual failure and disease penetrance was reported to be higher when m.11778G>A and m.14484T>C mutations existed on J2 and J1 haplogroups, respectively, whereas the same is true when m.3640G>A is present in haplogroup K. It was also observed that haplogroup H reduce the risk of disease manifestation in individuals with m.11778G>A mutations.[8] Similarly, two independent studies on Asian populations also showed that the haplogroups M7b1′2 and G increase visual failure and varied disease penetrance in the Chinese population with the m.11778G>A mutation, whereas M8a might confer a protective role with reduced disease penetrance.[9] The haplogroup B5a1 in South Asian populations has been reported to influence the expression of LHON in families with the m.11778G>A mutation.[22] A major contributor to the association of the mtDNA haplogroups (J1, J2, M7b1) with LHON expression in families harboring the m.11778G>A and m.14484T>C mutations also relates to specific combinations of amino acid changes (L236I-F19L and L236I-D171N-V356M) in the cytochrome b gene.[6]

There are very few studies that have focused on primary mtDNA LHON mutations in Indian patients,[23–26] and none of these have analyzed the association between mtDNA haplogroup and clinical expression. High-resolution genetic studies revealed an in situ origin for several deep-rooted mtDNA lineages in India, suggesting that Indian populations are genetically unique and display the second highest genetic diversity after the African population.[27] This genomic complexity, encouraged by the practice of endogamy, language shifts, and sex-specific admixture over thousands of years, provides substantial challenges to the understanding of disease mechanisms and the implementation of personalized management plans. Furthermore, the accumulation of private mutations as a result of endogamy has resulted in numerous recessive diseases in Indian populations, which further increases the total disease burden of the country.[28,29] Given the potential influence of the mtDNA haplogroup on the clinical expression of LHON and the highly complex genetic architecture of India, we examined mtDNA haplogroup distribution in 543 individuals from 64 pedigrees harboring the m.11778G>A mutation. Detailed clinical, genetic (including complete mtDNA sequencing), and phylogenetic analysis was subsequently undertaken to determine potential modifiers of m.11778G>A-related LHON in Indian patients.

## Materials and Methods

### Patients

Patients with optic neuropathy who were clinically suspected for LHON were recruited from the Department of Neuro-Ophthalmology, Sankara Nethralaya Chennai, India; the National Institute of Mental Health and Neurosciences, Bengaluru, India; and Nizam's Institute of Medical Sciences, Hyderabad, India, from 2006 to 2015. All of the clinical investigations were conducted by an expert panel of opthalmologists and neurologists at the above respective hospitals. The patients were thoroughly investigated based on the presentation of primary features, including acute, gradual, and progressive loss of central vision; color vision defect and centro ceocal scotomas; visual acuity measurement; and optic atrophies. Ophthalmic examinations, including an evaluation by snellens chart, visual acuity measurement, slit lamp biomicroscopy, indirect ophthalmology, Humphrey permetry analysis, and visual field testing, were performed in all of the patients with good fixation. The degree of visual impairment was defined according to the visual acuity as follows: normal >0.3, mild 0.3–0.1; moderate <0.1–0.05, and severe <0.05–0.02.

The family members of the 64 probands, recruited through clinics, participated in this study. The spouses of matrilineal members and children of male members were excluded from the study except in cases of consanguinity. Siblings were included only if there was one affected individual and the mother harbored the m.11778G>A. The age of the individuals were recorded at the time of sample collection, and the data regarding actual age of disease onset and start of symptoms were retrieved. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Ethical Committees of all the participating institutes. Informed written consent was obtained from all of the individuals who participated in this study prior to the collection of blood samples.

### Genetic Analysis

DNA was extracted from blood samples using standard protocol.[30] Complete mitochondrial DNA of all the probands were amplified using 24 sets of primers, and the amplicons were subjected to sequencing of both forward and reverse strands, seperately,[31,32] using the ABI BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Extended products were precipitated with ethanol:sodium acetate, washed with 70% alcohol, gently dried and dissolved with Hi-Di formamide, and analyzed in the ABI 3730 DNA Analyzer (Applied Biosystems). All of the 48 (24 forward and 24 reverse) sequence electropherograms were edited using sequence analysis software and assembled with the revised Cambridge Reference Sequence (NC_012920)[33,34] using AutoAssembler software (Applied Biosystems). We assessed for heteroplasmy of the m.11778G>A mutation by both Sanger sequencing and PCR-restriction fragment length polymorphism analysis as described previously.[22] All of the maternal relatives (543 individuals) were genotyped for the variant m.11778G>A and assessed for heteroplasmy. Samples with a mutation load greater than 90% were considered to be homoplasmic. The sequence (mtDNA) of the 64 index patients included in the present study has been submitted to the GenBank database with the following accession numbers: JQ446407–JQ446410, JX462680, JX462681, JX462683–JX462687, JX462689, JX462691–JX462696, JX462698, JX462700–JX462704, JX462706–JX462711, JX462713,

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JX462714, JX462716–JX462739, JX508849–JX508853, KX146833–KX146835.

### Data Analysis

All mismatched nucleotide positions were noted and searched in the human mitochondrial genome databases, such as 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 data obtained were also compared with 300 ethnically matched controls. Novelty and potential pathogenicity for the nonsynonymous private variants were analyzed using the method reported previously.[35] All of the data analyses were carried out using MEGA5 (www.megasoftware.net) to check for the conservation of amino acid in mitochondrial-encoded protein subunits and nucleotide conservation of mitochondrial ribosomal RNA (*MT-rRNA*). The online tools PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2) and PROVEAN (http://provean.jcvi.org) were used to assess the biological effects of the amino acid substitution on the structure and function of mitochondrial proteins. In addition, we used a reported system for determining the evolutionary conservation of mitochondrial transfer RNA (*MT-tRNA*) point mutations[36] and pathogenicity.[37]

### Haplogroup Assignment and Comparison

Phylogenetic analysis was performed based on the mtDNA variations of the LHON patients with the m.11778G>A mutation and available literature (mtDNA Tree Build 17, www.phylotree.org). The haplogroup information obtained from this study was compared with 7518 individuals from 138 endogamous populations (our published and unpublished data).[38–41]

### Statistical Analysis

Group comparisons were performed using the $\chi^2$ test, Student's *t*-test, and one-way analysis of variance, with 95% confidence intervals being determined as appropriate. Binary logistic regression analysis was performed to determine the effects of the variables (sex, heteroplasmy, and haplogroup) on the phenotypic expression of LHON using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). An analysis of variance was used to compare age at onset in the different haplogroups.

## Results

### Clinical Evaluation of LHON Families With the m.11778G>A Mutation

A total of 64 families included in this study were selected after the genetic screening of 219 families who were suspected to have LHON. These 64 families consisted of 543 maternally related individuals (146 affected and 397 unaffected) carrying the m.11778G>A mutation (Table 1). Total penetrance was 146 of 543 individuals (27.07%) who showed the clinical phenotype of LHON and loss of vision. The affected individuals had painless bilateral loss of central vision along with scotomas (central and cecocentral) and optic disc atrophy (Fig.). The visual acuity status of all the probands are given in Supplementary Table S1. Family members who were carrying the primary LHON m.11778G>A mutation but not showing any symptoms of visual impairment at the time of sample collection were considered as unaffected individuals. Among the individuals who carried the m.11778G>A mutation, 295 (54.3%) were males and 248 (45.5%) were females (Table 2).

**Table 1.** Mitochondrial Haplogroup Distribution of Affected and Unaffected Individuals With m.11778G>A Mutation

| Haplogroup | Unaffected, *n* | Affected, *n* (%) | Total, *n* |
|---|---|---|---|
| I1 | 11 | 1 ( 8.34) | 12 |
| M | 5 | 1 (20.00) | 6 |
| M10a | 4 | 1 (20.00) | 5 |
| M13a | 9 | 3 (25.00) | 12 |
| M18 | 9 | 3 (25.00) | 12 |
| M1a | 3 | 1 (25.00) | 4 |
| M2 | 31 | 13 (29.55) | 44 |
| M25 | 7 | 1 (12.50) | 8 |
| M30 | 45 | 18 (28.57) | 63 |
| M4′64 | 11 | 3 (21.43) | 14 |
| M33d | 8 | 3 (27.27) | 11 |
| M34a | 4 | 2 (33.33) | 6 |
| M35a | 7 | 2 (22.22) | 9 |
| M39b | 7 | 3 (30.00) | 10 |
| M3 | 31 | 18 (36.73) | 49 |
| M42 | 13 | 5 (27.77) | 18 |
| M45a | 9 | 2 (18.18) | 11 |
| M5 | 22 | 8 (26.66) | 30 |
| M52a | 9 | 4 (30.77) | 13 |
| M65a | 11 | 5 (31.25) | 16 |
| M66 | 17 | 4 (19.05) | 21 |
| M6a | 6 | 1 (14.29) | 7 |
| R | 5 | 1 (16.67) | 6 |
| R30 | 14 | 8 (33.36) | 22 |
| R5a2 | 12 | 5 (29.41) | 17 |
| R7a | 9 | 4 (30.77) | 13 |
| R8a1a | 4 | 2 (33.33) | 6 |
| T | 9 | 2 (18.11) | 11 |
| U1a3 | 3 | 1 (25.00) | 4 |
| U2 | 25 | 7 (21.8) | 32 |
| U4a | 4 | 1 (20.00) | 5 |
| U5 | 9 | 4 (30.77) | 13 |
| U7a | 13 | 6 (31.58) | 19 |
| X2 | 11 | 3 (21.4) | 14 |
| **Total** | **397** | **146** | **543** |

The penetrance of the disease was much higher in males when compared with females, with 37.9% of males (112/295) who carried the m.11778G>A mutation developed blindness, whereas only 13.7% of females (34/ 248) presented with loss of vision. This observation was in accordance with previous reports from European and Chinese studies,[8,9] suggesting that the sex factor has a strong influence with a 3.9-fold increased risk of visual failure in males when compared with females who harbor the m.11778G>A mutation in the present study ($P < 0.0001$; odds ratio = 3.92; 95% confidence interval = 2.548–6.032). A total of 13 of 64 LHON families possessed at least one individual with heteroplasmy, and they were classified as heteroplasmic families. The mtDNA heteroplasmy was associated with a 0.39-fold reduced risk of visual failure compared to homoplasmic pedigrees ($P < 0.0003$, odds ratio = 0.39, 95% confidence interval = 0.2385–0.6491). A total of 19 childhood (age < 18 years) LHON probands with the m.11778G>A mutation were identified in the present study. The mean age of onset for 146 affected individuals from 64 families with the m.11778G>A mutation was 22.87 ± 6.49 (median 23), and >95% of the patients affected were younger than the age of 40 years. The mean age of disease onset and vision loss in the affected males was 22.06 ± 6.1 (median 23 years) and in the affected females was 25.37 ± 6.89 (median 26 years). However, no significant difference in the age of onset among different mtDNA haplogroups was observed (data not shown).

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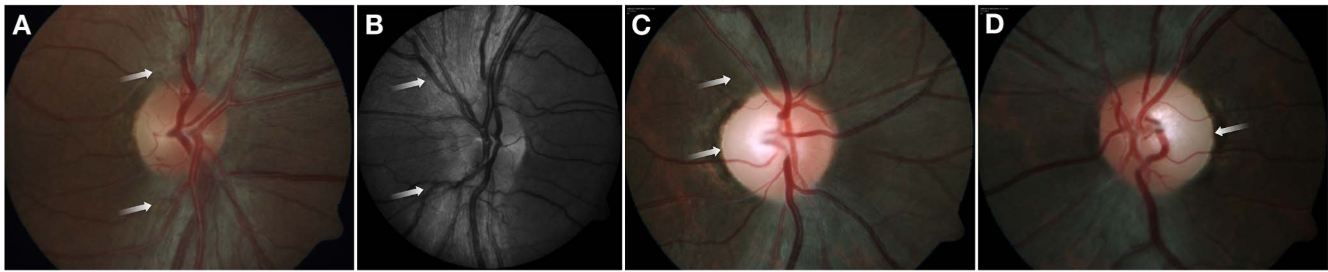


**Figure.** Fundus picture of two patients with m.11778G>A mutation in the *MT-ND4* gene. Patient 1: (**A**) oculus dextrus–acute onset vision loss, optic disc appearance showing nerve fiber layer edema and dilated capillaries (*arrow*); (**B**) oculus sinister–red free photo of disc showing nerve layer edema (*arrow*). Patient 2: (**C**) oculus dextrus–subacute presentation showing temporal pallor of disc and persisting nerve fiber layer edema (*arrow*), (**D**) oculus sinister–fellow eye showing significant disc temporal pallor as a result of loss of nerve fiber in papillomacular bundle region (*arrow*).

## Haplogroup Distribution Among Individuals With the m.11778G>A Mutation

The mtDNA haplogroup was constructed based on the mutations observed in the complete mtDNA sequences of all the probands from 64 families who possessed the m.11778G>A mutation. The haplogroup distribution of LHON families is illustrated in Table 1. Individuals who carried the primary LHON mutation m.11778G>A belonged to 34 different mitochondrial haplogroups. The existence of diverse mtDNA haplogroup among the families carrying the m.11778G>A mutation suggests that these families are not maternally related. The haplogroup distribution pattern showed that 67.7% of the individuals belonged to the macrohaplogroup M and were distributed in 21 subhaplogroups. The other major haplogroups were R (11.7% including its five subhaplogroups) and U (16.5% including its five subhaplogroups). A small proportion of the samples belonged to haplogroups I (2.2%), T (2.0%), and X (2.5%; Table 1). Interestingly, we did not find any of the studied samples belonging to haplogroup J. Total haplogroup distribution showed that 68.4% of the affected males and 67.6% of the affected females belonged to haplogroup M, with a high frequency of subhaplogroups M2, M3, and M30 (Table 2). The distribution of major haplogroups among both heteroplasmic and homoplasmic families were similar except in haplogroups I, T, and X, which were all homoplasmic. These families belonged to diverse ethnic and linguistic groups inhabited in different geographical regions of India (Supplementary Fig. S1).

**Table 2.** The Number of Male and Female Individuals Who Carry the m.11778G>A Mutation

| | Males, *n* | | Females, *n* | |
|---|---|---|---|---|
| **Haplogroup** | **Affected** | **Unaffected** | **Affected** | **Unaffected** |
| I1 | 1 | 4 | 0 | 7 |
| M | 1 | 3 | 0 | 2 |
| M10a | 1 | 2 | 0 | 2 |
| M13a | 3 | 6 | 0 | 3 |
| M18 | 1 | 4 | 2 | 5 |
| M1a | 1 | 0 | 0 | 3 |
| M2 | 9 | 13 | 4 | 18 |
| M25 | 1 | 3 | 0 | 4 |
| M30 | 15 | 24 | 3 | 21 |
| M4′64 | 2 | 4 | 1 | 7 |
| M33d | 2 | 3 | 1 | 5 |
| M34a | 2 | 2 | 0 | 2 |
| M35a | 2 | 4 | 0 | 3 |
| M39b | 2 | 3 | 1 | 4 |
| M3 | 12 | 17 | 6 | 14 |
| M42 | 4 | 5 | 1 | 8 |
| M45a | 2 | 6 | 0 | 3 |
| M5 | 7 | 9 | 1 | 13 |
| M52a | 3 | 5 | 1 | 4 |
| M65a | 3 | 5 | 2 | 6 |
| M66 | 4 | 9 | 0 | 8 |
| M6a | 1 | 2 | 0 | 4 |
| R | 1 | 2 | 0 | 3 |
| R30 | 6 | 6 | 2 | 8 |
| R5a2 | 3 | 4 | 2 | 8 |
| R7a | 4 | 5 | 0 | 4 |
| R8a1a | 2 | 1 | 0 | 3 |
| T | 1 | 3 | 1 | 6 |
| U1a3 | 1 | 1 | 0 | 2 |
| U2 | 4 | 14 | 3 | 11 |
| U4a | 1 | 1 | 0 | 3 |
| U5 | 3 | 3 | 1 | 6 |
| U7a | 4 | 6 | 2 | 7 |
| X2 | 3 | 4 | 0 | 7 |
| **Total** | **112** | **183** | **34** | **214** |

## MtDNA Haplotype Analysis of Individuals With LHON

The haplotype distribution from the present data showed 64 independent mutational events among LHON families in India. The phylogenetic tree (Supplementary Fig. S2A, S2B) illustrates the haplogroup distribution of all the 64 LHON families. The complete mtDNA sequence of the patients of same haplogroups/subhaplogroups carrying the mutation m.11778G>A also differ from each other by several variations (Supplementary Fig. S2A, S2B). We also compared the control region sequence of the LHON patients with 7518 individuals belonging to 138 endogamous populations, representing all the major linguistic families inhabited in different regions of India (data not shown). The occurrence of similar control region sequence motifs in random population samples further supports the independent occurrence of the m.11778G>A mutation event in these pedigrees. Furthermore, we observed that the distribution of different haplogroups in 543 individuals with m.11778G>A and 7518 individuals from 138 endogamous populations were almost similar in all the haplogroups except M30, M3, and U2 (Table 3).

## Complete mtDNA Analysis From LHON Probands

Complete mtDNA sequencing analysis of all 64 probands revealed 597 variations across the mitochondrial genome. Of the total variations observed, 17 were novel (neither reported in databases nor observed in controls), of which 2 were

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TABLE 3. Mitochondrial Haplogroup Comparison Between the Population Individuals and LHON Families With the m.11778G>A Mutation

| Haplogroup | No. of subjects (%) | Population Individuals (%) |
|---|---|---|
| I1 | 12 (2.20) | 10 (0.13) |
| M | 6 (1.10) | – |
| M10a | 5 (0.92) | 34 (0.45) |
| M13a | 12 (2.20) | 8 (0.11) |
| M18 | 12 (2.20) | 41 (0.54) |
| M1a | 4 (0.73) | 9 (0.12) |
| M2 | 44 (8.07) | 541 (7.14) |
| M25 | 8 (1.47) | 293 (3.86) |
| M30 | 63 (11.56) | 192 (2.55) |
| M4′64 | 14 (2.57) | 278 (3.67) |
| M33d | 11 (2.02) | 411 (5.42) |
| M34a | 6 (1.10) | 243 (3.21) |
| M35a | 9 (1.65) | 39 (0.51) |
| M39b | 10 (1.83) | 7 (0.09) |
| M3 | 49 (8.99) | 219 (2.89) |
| M42 | 18 (3.30) | 92 (1.21) |
| M45a | 11 (2.02) | 3 (0.03) |
| M5 | 30 (5.50) | 603 (7.95) |
| M52a | 13 (2.39) | 93 (1.23) |
| M65a | 16 (2.94) | 57 (0.75) |
| M66 | 21 (3.85) | 23 (0.30) |
| M6a | 7 (1.28) | 114 (1.50) |
| R | 6 (1.10) | 1291 (17.3) |
| R30 | 22 (4.04) | 428 (5.65) |
| R5a2 | 17 (3.12) | 713 (9.41) |
| R7a | 13 (2.39) | 523 (6.90) |
| R8a1a | 6 (1.10) | 27 (3.64) |
| T | 11 (2.02) | 8 (0.11) |
| U1a3 | 4 (0.73) | 12 (0.16) |
| U2 | 32 (6.24) | 151 (1.99) |
| U4a | 5 (0.92) | 17 (0.22) |
| U5 | 13 (2.39) | 36 (0.47) |
| U7a | 19 (3.49) | 34 (0.45) |
| X2 | 14 (2.57) | 6 (0.08) |
| Others | – | 962 (12.79) |
| **Total** | **543** | **7518** |

nonsynonymous and 15 were synonymous. The private nonsynonymous variations observed in the haplogroups are one of the most important factors that influences the effect of primary mutations and may result in variable clinical expression.[8,9] Hence, we analyzed the private and nonsynonymous variants observed in each proband and found 39 nonsynonymous variants and 6 variants in *MT-tRNA* genes of the probands (Table 4).

## DISCUSSION

More than 95% of LHON is caused by the following three mtDNA ND mutations: m.3460G>A, m.11778G>A, and m.14484T>C. Of these, m.11778G>A is the most common mutation among different LHON cohorts with the only exception being in Canada, where the m.14484T>C mutation is reported to be the major cause.[42,43] In European LHON patients, the m.11778G>A and 14484T>C mutations were reported to be more predominant in the haplogroup J[13,20] background with an increased clinical penetrance when present on the J2 subhaplogroup.[8] A similar study in Chinese LHON families reported increased penetrance with mtDNA haplogroup M7b1′2.[9] Given the potential influence of the mtDNA haplogroup on clinical expression, we analyzed the distribution of mtDNA haplogroup within Indian populations and investigated the possible influence of mtDNA haplogroup in the disease phenotype.

Detailed clinical and genetic characterizations of 64 families (543 individuals) harboring the m.11778G>A mutation were performed. In the present study, the frequency of m.11778G>A was 29.22% (64/219), which is much higher than previous reports of the m.14484T>C mutation (4.2%).[26] In accordance with the earlier reports, sex bias and heteroplasmy were strong factors associated with visual loss in Indian LHON with males 3.9 times more at risk of developing blindness than females. In the present cohort, at least one individual heteroplasmic for the m.11778G>A mutation was identified in 20.3% of the families, whereas no heteroplasmy was observed in our previous study of families harboring the m.14484T>C mutation.[26] The heteroplasmic families showed a 0.39-fold reduced risk of visual failure compared to families with the homoplasmic mutation. In the present study, 19 (29.7%) of 64 LHON probands with the m.11778G>A mutation experienced childhood (<18 years) age of onset. A previous study has shown that childhood LHON exhibits a number of unique clinical features distinct from adult forms of the disease.[44] However, we could not correlate any specific differences in the clinical features of LHON between childhood and adult in our cohort. LHON is a primary mtDNA disorder with some symptoms overlapping with autosomal dominant optic atrophy, blindness caused as a result of pathogenic mutations within the nuclear gene *OPA1*. In the majority of cases, LHON mutations lead to isolated optic nerve atrophy, and occasionally patients exhibit additional neurological symptoms.[45–47] However, no such additional clinical features were observed in our cohort.

The dissection of the matrilineal genetic structure of our cohort revealed that individuals with the primary LHON mutation (m.11778G>A) belong to 31 distinct mitochondrial haplogroups (Supplementary Figs. S2A, S2B). A detailed analysis of the complete mtDNA sequence data of all 64 samples revealed 62 different haplotypes. Because the primary LHON mutation m.11778G>A has been detected in distantly related ethnic populations with distinct mtDNA haplotypes, different sets of single nucleotide polymorphisms (SNPs) must have similar effects as either predisposing factors for the generation or as positive modifiers of primary LHON mutations. Of the 39 nonsynonymous and 6 *MT-tRNA* private variants observed in the families, we could not detect specific variants that might have a secondary role in determining disease penetrance. Previous studies using transmitochondrial cybrids demonstrated that inherited basal differences in an oxidative phosphorylation capacity might contribute toward the bioenergetics threshold for disease penetrance.[45] The functional changes induced by specific haplogroups might become more detrimental when the cell function is compromised, as is the case when mtDNA mutation is present or if exposed to specific environmental and/or nuclear backgrounds.[48,49] Another study also showed that some variants might be deleterious or beneficial depending on the haplogroup and environmental background.[50] Interactions between primary and secondary mutations (m.11778G>A and m.14502T>C) have also been proven to severely affect complex I activity when compared with individuals with a single mutation.[51] In this context, we also observed one pedigree carrying m.14502T>C along with m.11778G>A and a M10a haplogroup background, suggesting that these variants might have different effects depending on their environmental, nuclear, and haplogroup backgrounds.

In conclusion, this is the first study to investigate the association between the m.11778G>A mutation and mtDNA haplogroup in Indian LHON patients. The distinct set of sequence variants observed in the Indian pedigrees suggests that the m.11778G>A mutation might have arisen indepen-

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TABLE 4. The Private Nonsynonymous and *mt-tRNA* Variants Observed in LHON Probands With m.11778G>A.

| LHON Pedigree | Haplogroup | Nucleotide Variant | Gene | Amino Acid Change | Reported Population Context | Disease Association | Conservation | Polyphen-2 | Provean |
|---|---|---|---|---|---|---|---|---|---|
| P12 | M18a | m.4231A>G | *MT-ND1* | I309V | Yes | No | No | Benign | Neutral |
| | | m.4240T>C | *MT-ND1* | S312P | Yes | No | No | Possibly damaging | Neutral |
| | | m.15156A>G | *MT-CYB* | Q137R | Yes | No | No | Probably damaging | Deleterious |
| P14 | U2b2 | m.3310A>G | *MT-ND1* | P2S | Yes | Yes | No | Benign | Neutral |
| | | m.13678C>T | *MT-ND5* | P448S | No | No | No | Benign | Deleterious |
| P16 | M13a | m.14737A>G | *MT-tRNA Glu* | – | Yes | No | No | – | – |
| | | m.3644T>C | *MT-ND1* | V113A | Yes | Yes | No | Benign | Deleterious |
| P21 | M42b1 | m.8462T>C | *MT-ATPase8* | Y33H | Yes | No | No | Probably damaging | Deleterious |
| | | m.6160T>A | *MT-COI* | M86T | Yes | No | No | Probably damaging | Deleterious |
| P27 | M52a | m.11015A>G | *MT-ND4* | S86G | Yes | No | No | Benign | Neutral |
| P30 | M18b | m.3368T>G | *MT-ND1* | M21T | Yes | No | No | Benign | Neutral |
| P31 | M2a1 | m.6267G>A | *MT-COI* | A122T | Yes | Yes | No | Benign | Neutral |
| P34 | M2a3 | m.9022G>A | *MT-ATPase6* | A166T | Yes | No | No | Probably damaging | Deleterious |
| P39 | M65a1 | m.3316G>A | *MT-ND1* | A4T | Yes | Yes | No | Benign | Neutral |
| P40 | M3a1 | m.15624 Tins | *MT-CYB* | – | Yes | No | No | – | – |
| P43 | R30a | m.9142G>A | *MT-ATPase6* | V206I | Yes | No | No | Benign | Neutral |
| P45 | M2b1 | m.8349C>T | *MT-tRNA Lys* | – | Yes | No | No | – | – |
| | | m.11016G>A | *MT-ND4* | S86N | Yes | No | No | Benign | Neutral |
| P47 | M65a | m.13708G>A | *MT-ND5* | A458T | Yes | Yes | No | Benign | Neutral |
| P49 | M33d | m.13789T>C | *MT-ND5* | Y485H | Yes | No | No | Probably damaging | Neutral |
| P50 | M* | m.5279C>A | *MT-ND2* | F270L | Yes | No | No | Benign | Neutral |
| | | m.8307A>G | *MT-tRNA Lys* | – | Yes | No | No | – | – |
| P53 | M52a1 | m.11015A>G | *MT-ND4* | S86G | Yes | No | No | Benign | Neutral |
| P57 | U7a | m.4638T>C | *MT-ND2* | I57V | Yes | No | No | Benign | Neutral |
| P62 | U2b1 | m.4500T>C | *MT-ND2* | S11P | Yes | No | No | Benign | Neutral |
| | | m.8743G>A | *MT-ATPase6* | V73M | Yes | No | No | Benign | Neutral |
| P64 | M5a'd | m.7521G>A | *MT-tRNA Asp* | – | Yes | No | No | – | – |
| | | m.8412T>C | *MT-ATPase8* | M16T | Yes | No | No | Probably damaging | Deleterious |
| P65 | M2b1 | m.14059A>G | *MT-ND5* | I575V | Yes | No | No | Benign | Neutral |
| P67 | U5b2a1a2 | m.4695T>C | *MT-ND2* | F76L | Yes | No | No | Benign | Neutral |
| P69 | M3c1b | m.4435A>G | *MT-tRNA Met* | – | Yes | Yes | No | – | – |
| | | m.6366G>A | *MT-COI* | V155I | Yes | No | No | Benign | Neutral |
| P71 | R5a2a | m.6681T>C | *MT-COI* | Y260H | No | No | No | Benign | Neutral |
| P73 | R30b | m.8584T>C | *MT-ATPase6* | A20T | Yes | No | No | Benign | Neutral |
| | | m.14000T>A | *MT-ND5* | L555Q | No | No | No | Probably damaging | Deleterious |
| P77 | M5c1 | m.12811T>C | *MT-ND5* | Y159H | Yes | No | No | Benign | Neutral |
| | | m.15884G>A | *MT-CYB* | A380T | Yes | No | No | Probably damaging | Neutral |
| P78 | M35b | m.9861T>C | *MT-COIII* | F219L | Yes | No | No | Benign | Neutral |
| | | m.13145G>A | *MT-ND5* | S270N | Yes | No | No | Benign | Neutral |
| P79 | X2P1 | m.12358A>G | *MT-ND5* | T8L | Yes | No | No | Benign | Neutral |
| P80 | X2P1 | m.7119G>A | *MT-COI* | D406N | No | No | No | Benign | Neutral |
| P81 | M35a | m.9181A>G | *MT-ATPase6* | S219G | Yes | No | No | Probably damaging | Deleterious |
| P82 | M45a | m.5139A>C | *MT-ND2* | S224R | No | No | No | Probably damaging | Deleterious |
| | | m.15119G>A | *MT-CYB* | A125T | Yes | No | No | Benign | Neutral |
| P83 | C | m.4216T>C | *MT-ND1* | Y304H | Yes | Yes | No | Benign | Neutral |
| | | m.11534T>C | *MT-ND4* | Y259H | Yes | No | No | Probably damaging | Deleterious |

dently in different mitochondrial haplogroup backgrounds. Furthermore, we did not find any association between the m.11778G>A mutation and specific haplogroup. Variable penetrance of LHON against different Indian haplogroup backgrounds was observed, indicating a possible influence on the clinical expression of the disease. However, an evaluation of additional patient cohorts is necessary to gain further insights into the m.11778G>A mutation and its haplogroup association with LHON in Indian populations.

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